MS – thesis Maí 2009

Effect of dry period diets varying in energy density on health and performance of periparturient dairy cows

A study of dry matter intake, lactation performance, fertility, blood parameters and liver condition

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Clarification of contribution

I hereby declare that the statistical analysis and writing of this thesis and two manuscripts is my work under the supervision and assistance of my advisors Jóhannes Sveinbjörnsson og Grétar Hrafn Harðarson.

The experiment that the thesis and two manuscripts are based on was performed at the research farm Stóra- Ármót. Daily management was in the hands of local staff at Stóra-Ármót. I however visited the research farm for a few days and monitored all elements of the management and participated while I stayed there. The feed was analysed at the laboratory in Agricultural University of Iceland under the supervision of Tryggvi Eiríksson. I prepared the samples for analysing and participated a little in every analytic method. Þorsteinn Ólafsson assisted in estimating time of ovulation. The liver samples were prepared under the supervision of Sverrir Harðarson, he taught me how to take microscopic pictures of the samples. I took the microscopic pictures to Matís in Akureyri were I was able to estimate liver fat in the computer program Leica QWin under the supervision of Jónína Jóhannsdóttir. The blood samples were sent to Denmark and analysed under the supervision of Torben Larsen at the Aarhus University.

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Dagsetning og staður Berglind Ósk Óðinsdóttir

Abstract

Effect of feed with different energy levels in the close-up dry period (3-0 weeks prepartum) on different production and health parameters were examined around parturition of 30 Icelandic cows and heifers in individual tie stalls. Dry matter intake (DMI) was registered, lactation performance, i.e. milk yield and milk composition were measured and analyzed, ovarian activity was estimated by measuring concentration of the hormone progesterone in milk. The metabolic status of the cows was monitored by collection and analysis of liver biopsies and blood samples. Glucose, BHB (β-hydroxybutyrate), NEFA (Non-esteriffied fatty acids), GLDH (Glutamate dehydrogenase), AST (Aspartat aminotransferase), GGT (Gamma glutamyl transferase), ICDH (Isocitrate dehydrogenase) were analyzed from blood and liver fat was estimated under a microscope. Ketolac® test was used to estimate the level of BHB in milk. DMI prepartum was higher for cows receiving higher energy levels in feed but they did not maintain significantly higher DMI into the lactation, increased energy prepartum gave lower fat- and urea concentration in milk. Parity had the greatest effects on ECM, protein and lactose in milk. Treatments did not significantly affect ovarian activity, ketone bodies in milk as estimated by Ketolac test or liver fat infiltration. Glucose concentration in blood increased significantly with increased energy level, there was also a significant effect of periods and parity. Treatment did not affect BHB or NEFA concentration significantly. BHB concentration was highest in the first three weeks postpartum and heifers had lower BHB concentration than older cows. NEFA concentration was highest around parturition. Treatments affected GLDH enzyme concentration significantly, GLDH increased with increased energy level in diet. Treatments did not affect AST, GGT or ICDH significantly.

Keywords: dry period nutrition, periparturient period, dry matter intake, lactation performance, ovarian activity, non esterified fatty acids, β-hydroxybutyrate, fatty liver, liver enzyme.

Abbreviation key: DL = low energy level; DM = medium energy level; DH = high energy level; BHB = β-hydroxybutyrate; NEFA = Non-esterified fatty acids; GLDH = Glutamate dehydrogenase; AST = Aspartat aminotransferase; GGT = Gamma glutamyl transferase; ICDH = Isocitrate dehydrogenase

Yfirlit

Markmið rannsóknarinnar var að skoða áhrif mismunandi fóðrunar seinustu þrjár vikur fyrir burð á marga mismunandi þætti kringum burðinn. Þurrefnisát var mælt, nyt var mæld og mjólkin efnagreind, frjósemi með því að greina styrk prógesteróns í mjólk og efnaskipti líkamans með því að taka lifrar og blóðsýni. Glúkósi, BHB (β-hydroxybutyrate), NEFA (Non-esteriffied fatty acids), GLDH (Glutamate dehydrogenase), AST (Aspartat aminotransferase), GGT (Gamma glutamyl transferase), ICDH (Isocitrate dehydrogenase) var greint í blóði og fita í lifur metin undir smásjá. Auk þess sem BHB í mjólk var greind. Fóðrunin hafði marktæk áhrif á þurrefnisát fyrir burð, þar sem orkumeira fóður gaf meira át. Ekki fundust marktæk áhrif tilraunameðferða á þurrefnisát eftir burðinn. Það var ekki marktækur munur á þungabreytingu kúnna milli meðferða. Mjólkurmagn eða efnainnihald mjólkur varð ekki fyrir marktækum áhrifum af mismunandi fóðrun fyrir burð, að undanskildu fitu- þvagefnisinnihaldi mjólkur sem lækkaði með orkumeiri fóðrun. Mjaltaskeið hafði marktæk áhrif á nyt, prótein- og mjólkursykurinnihald mjólkur. Meðferðirnar höfðu ekki marktæk áhrif á frjósemi. Meðferðirnar höfðu ekki marktæk áhrif á niðurstöður mjólkurprófsins eða fitu í lifur. Glúkósi jókst marktækt í blóði eftir því sem kýrnar fengu orkuríkara fóður, einnig höfðu tímabil og mjaltaskeið marktæk áhrif. Meðferðirnar höfðu ekki marktæk áhrif á magn BHB eða NEFA í blóði. Magn BHB í blóði var mest fyrst eftir burðinn og einnig höfðu eldri kýr meira BHB í blóði en kvígur. NEFA í blóði var mest í kringum burð. Meðferðirnar höfðu marktæk áhrif á GLDH magn, sem jókst með aukinni orku í fóðri. Meðferðirnar höfðu ekki marktæk áhrif á AST, GGT og ICDH.

Acknowledgements

I would like to thank Mrs. Svala Sigurgeirsdóttir for daily management of the experiment, Mr. Tryggvi Eiríksson for chemical analysis of feed stuff and Mr Torben Larsen for chemical analysis of blood. I am also thankful to Mr. Sverrir Harðarson for embedding the liver samples on glass and Mrs. Jónína Jóhannsdóttir at Matís for assisting with the computer program Leica QWin. I also want to thank Þórunn Edda Bjarnadóttir for technical assistance while structuring the thesis.

I would like to give thanks to my advisors Jóhannes Sveinbjörnsson and Grétar Hrafn Harðarson, Department of land and animal resources, LbhÍ for good guidance and advice during my work.

I want to thank my partner Sigtryggur Veigar Herbertsson, my family and many friends for moral support and encouragement during my studies.

Financial support from the Agricultural Productivity fund and fund of Blikastaðir is gratefully acknowledged.

Last but not least I want to dedicate this thesis to my son Óskar Óðinn who has shown a lot of patience during my studies. You can do whatever you set your mind to; I hope I have given you a good example. With love from me to you.

Contents

Manuscript I submitted to Icelandic Agricultural Sciences: Effect of dry period nutrition on, dry matter intake, lactation performance and fertility in dairy cattle. By Berglind Ósk Óðinsdóttir, Grétar Hrafn Harðarson and Jóhannes Sveinbjörnsson

Manuscript II submitted to Icelandic Agricultural Sciences: Effect of feed in the dry period of Icelandic dairy cows on blood parameters and liver condition. By Berglind Ósk Óðinsdóttir, Grétar Hrafn Harðarson, Jóhannes Sveinbjörnsson and Torben Larsen.

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1. Introduction

The Icelandic cow is relatively small and milked on average 5500 kg per cow in the year 2007. Icelandic dairy farmers have been increasing the milk yield with better feeding and management in recent years. The Icelandic cow milked on average 4664 and 5079 kg in the year 2000 and 2003, respectively. In the year 2007 10 farms in Iceland had over 7000 liters in average yield per cow and 134 farms had over 6000 liters.

This thesis is based on a feeding experiment that was performed on Icelandic dairy cows. The aim of the experiment was to see how three differently concentrated diets prepartum affected dry matter intake, lactation performance, fertility and health parameters. In addition to the thesis two manuscripts have been written. Manuscript I covers the results of dry matter intake, lactation performance and fertility, Manuscript II covers the results of blood parameters and liver condition.

1.1 Lactation

The mammary gland starts to develop in fetal live, it also undergoes a rapid development during puberty and most importantly, during pregnancy. When milk synthesis has started, milk production is sustained by hormonal signals elicited either by the suckling of the newborn calf or by milking of the mother (Sjaastad et al., 2003). Milk secretion is regulated both by hormonal concentration in blood and conditions in the alveoli, which represent the inner structure of the mammary gland. If the cow is not suckled by the calf or milked, milk production will cease even if the hormonal changes normally connected to lactation are mimicked by injections. And without the necessary hormone concentration in the blood, milk production will gradually decline even if milking is continued (Sjaastad et al., 2003). Ingvartsen and Andersen, (2000) indicate that lactation is not just a function of the mammary gland but involves many physiological processes and body tissues. The endocrine system plays a pivotal role, but the nervous system and the immune system are also involved in the regulation of metabolism and nutrient partitioning (Ingvartsen and Andersen, 2000).

The lactation cycle can be divided in four periods, beginning of lactation (first 12 weeks postpartum), mid lactation (12-24 weeks postpartum), late lactation (25 weeks postpartum to dry off) and dry period. All periods have their characteristics regarding energy

metabolism. In the beginning of lactation, production is increasing but feed intake does not increase the same way, this period is characterized by degradation where the cow is in a negative energy balance. This period is often the hardest to manage. The next period, mid lactation is characterized by a metabolic equilibrium. Feed intake is now supposed to fulfill energy requirements and stop degradation. In the third period, late lactation, the cow is in a positive energy balance and can therefore build up if necessary for the next lactation (Harðarson and Ingvartsen, 2005). When the dry period starts the cow is supposed to have reached desirable body condition.

1.2 Dairy cattle feeding

Dairy cows are used all over the world for milk production; many researchers have therefore studied dairy cattle management. Before the 1980s the transition period (3 weeks prepartum to 3 weeks postpartum) was set aside by researchers because of the extreme variability in animal responses and frequent incidence of health problems (Drackley et al., 2006). This period is never the less very important because of the extreme hormonal changes and mobilization of body reserves that occurs. Dairy cattle nutrition must satisfy the need for maintenance, growth, reproduction, lactation and health. It is very important to formulate diets so that the dairy cows can achieve their maximal potential for milk production with minimal stress. In recent years the transition period has received increased attention (Grummer, 1995, Ingvartsen, 2006, Harðarson and Ingvartsen, 2005, McNamara et al., 2003, Doepel et al., 2002, Kokkonen et al., 2004). As pointed out by Drackley et al., (2006), progress in understanding the physiology in the transition period has led to improved understanding of nutrient requirements. In spite of that, optimal nutrition and feeding of periparturient cows are not completely resolved. It can be an advantage for cows to increase reserves in the dry period and adapt to high-energy rations to avoid the ill effects of rapidly changing to a high-energy ration postpartum (Holcomb et al., 2001). Rapid increase of concentrate allowances and high concentrated ration during early lactation can lead to disturbances of rumen function, digestive disorders and reduced dry matter intake (Kokkonen et al., 2004, Ingvartsen et al., 2001). But overfeeding prepartum where the cows become too fat may cause less production or increased risk of metabolic disorders postpartum (Holcomb et al., 2001). Dann et al., (2006) found that feeding early in the dry period can also affect energy balance and performance in the first days of lactation.

It has been speculated that intake in early lactation can be improved by increasing VFA absorption capacity in rumen, which would reduce VFA accumulation and drop in rumen pH (Ingvartsen, 2006, Keady et al., 2001). The benefits achieved through feeding concentrates prepartum are likely to be the result of microbial adaptation to a highly fermentable carbohydrate diet (Stockdale, 2006). Advances that include further understanding of descriptive chemistry of feed components and better models of how the rumen microbial population digests fibrous and nonfibrous carbohydrates and synthesize microbial proteins are very important (Drackley et al., 2006). The microbial population in the rumen consists of bacteria, protozoa and fungi. The two main bacterial groups are, cellulolytic bacteria that break down cellulose and hemicellulose, and amylolytic bacteria that brake down starch. When cows are fed diet high in NDF (i.e. fiber, mainly hemicellulose and cellulose) the cellulolytic bacteria is very active and the rumen environment is stable, but when the cows are fed diet high in starch (concentrate) the amylolytic bacteria populate and more starch is degraded which lowers the pH in the rumen. Low pH in rumen has a negative effect on the performance of the cellulolytic bacteria and therefore on roughage utilization. Diets high in easily degraded carbohydrates are favorable when nutritional demands are high as they are postpartum. It will give quicker fermentation and faster evacuation of feed from rumen and thereby increase DMI. Diets with higher ruminal carbohydrate availability fed in the prepartum period may adapt the microbial population to postpartum diets, promote ruminal papillae development, increase absorptive capacity of the rumen epithelium, and reduce lipolysis by increasing glucogenic precursors, and result in more nutrients supplied to the cow (Dann et al., 1999). This is consistent with Holcomb et al., (2001) suggesting that it may be advantageous for cows to adapt to high-energy rations during the dry period to avoid the ill effects of rapidly changing high-energy ration postpartum. But keeping in mind that overfeeding prepartum to the extent that cows become too fat may cause less production and higher changes of metabolic upsets postpartum (Holcomb et al., 2001).

1.3 Dry matter intake

Dry matter intake regulation can be divided in physical regulation and physiological regulation. Physical regulation includes rumen volume (litres), digestion speed (kg DM/time), and evacuation time of undigested feed from rumen (kg DM/time) (Harstad, 1994). Properties of the animal are important. Size, body condition and weeks in

pregnancy are the animal factors that have greatest impact on rumen volume. Big cows have more room for feed, and fat cows eat less which can increase fat mobilization and diseases that follow. Late in pregnancy rumen volume decreases because of increased uterus size. Properties of the feed are also important, easily digested feed passes faster which increases intake potential. Too easily digested diet, however, can have a negative effect on rumen environment and thereby reduce intake and contribute to other problems. Structure of the diet affects how full the rumen is, and small structured diet increases feed intake but can reduce cellulose digestion (Harstad, 1994).

Negative nutrient balance around parturition and in early lactation is common and is connected to the homeorhetic regulation. This regulation includes changes in plasma concentration of homeorhetic hormones and changes in tissue sensitivity and tissue response to homeostatic hormones, like insulin and catecholamines in tissue (Ingvartsen, 2006). Changes in selected hormones, tissue sensitivity and tissue response during pregnancy and early lactation can be viewed in Table 1. Homeorhesis is coordinated changes in the metabolism, necessary to support a physiological state, and adaption to a new equilibrium that takes place over days or weeks. Homeostasis is the regulation that maintains the equilibrium of the organism with the environment; a regulation that takes place from minute to minute (Ingvartsen, 2006). The homeorhetic regulation that makes it possible for the cows to maintain homeostasis, is believed to be of major importance for disease resistance in cows, particularly metabolically related production diseases (Ingvartsen, 2006).

Energy requirements for maintenance and pregnancy of dairy cattle increase considerably during the last month prepartum. During this time, feed intake typically decreases even more (Vandehaar et al., 1999). If energy density of the diet is constant in the dry period, cows will experience similar decline in energy intake in the last period of pregnancy. Researchers have established that declining dry matter intake just before parturition is a major factor for increasing body fat mobilization and thereby increased uptake of nonesterified fatty acids (NEFA) by liver and accumulation of triacylgycerol (TG) in liver (Drackley et al., 2006). It has been recommended in literature regarding transition cows that dairy producers should try to maximize DMI in the last three weeks prepartum to prepare cows to eat more right after calving and thereby reduce ketosis and other metabolic disorders (Grummer, 1995). Several researchers have focused on maximizing intake in the

dry period (Vandehaar et al., 1999, Doepel et al., 2002, Rabelo et al., 2003), when others have focused on limiting energy intake in the dry period (Holcomb et al., 2001, Agenas et al., 2003, Douglas et al., 2006) all to improve DMI postpartum.

Table 1. Changes in selected hormones, tissue sensitivity and tissue response during pregnancy and early lactation (modified from (Ingvartsen, 2006)).

	Mid pregnancy	Late pregnancy	Early lactation
Homeorhetic hormones			
Progesterone	+	(-)	-
Placental lactogen		+	-
Estrogen		+	-
Prolactin		(+)	+
Somatotropin		(+)	+
Glucocorticoids (cortisol)		(+)	+
Leptin	+	-/+	-
Homeostatic hormones			
Insulin		-/+	-
Glucagon			(+)
CCK and somatostatin	?	?	?
Parathyrodiea hormone		(+)	+
1,25-dihydroxyvitamin D_3		(+)	+
Calcitonin		(-)	-
Tissue sensitivity (except udder)			
Insulin	+	-	-
Catecolamines		+	+
Tissue response (except udder)			
Insulin		-	-
Catecolamines	-	+	+
Udder tissue			
Lactose synthesis			+
Milk-fat synthesis			+
Milk-protein synthesis			+
Liver tissue			
Gluconeogenesis		(+)	+
Ketogenesis		(+)	+
Adipose tissue			
De novo fat synthesis	+	-	-
Fatty acid esterification	+	-	-
Lipolysis		+	+
Muscular tissue			
Protein synthesis		-	-
Proteolysis		+	+
Glucose consumption		-	-
Fatty acid consumption		+	+
Ketone body consumption		+	+
Bone tissue			
Osteogenesis (bone formation)			?
Osteolysis (bone disintegration)			+

Dry matter intake increases postpartum and usually peaks in mid lactation, around or after 12 weeks postpartum, whereas milk yield typically peaks between 5 and 7 weeks postpartum. Ingvartsen and Andersen, (2000) suggest that differences in intake postpartum are affected by the diet fed during lactation but may also depend on prepartum feeding and the influence of fatness or BCS of periparturient animals. It has been suggested that reduced intake in late pregnancy is caused by physical compression of rumen from growing uterus, but it is probably not the only reason. Experiments where postrumenal infusions of nutrients have been applied indicate that metabolic factors play an important role in regulating intake (Ingvartsen and Andersen, 2000). The quality and composition of feed influence the metabolic response. Energy-dense diets decrease feed intake in some situations (Vandehaar et al., 1999). Other explanations for decreased DMI include differences in forage source, forage: concentrate ratio, rate of passage, and protein source (Dann et al., 1999). Composition of the concentrates is important, most concentrate diets have large ratio of barley and oats which contain large amounts of easily degraded starch, by replacing these easily degraded components of the concentrate with slower degrading starch such as maize, the concentrate would not have as large negative effect on rumen environment when feeding large amounts. As the amount of easily fermented carbohydrates increases, DMI may be reduced because of a possible accumulation of VFA in the rumen, resulting in lower pH and potential negative effects on fibre digestion.

Substitution effect is a term describing the negative effect of concentrate intake on roughage intake. Concentrate added in small amounts and or/to low quality roughage tends to be eaten mostly in addition to the roughage, i.e. the substitution effect is small. When concentrate is added in great amounts and/or to high quality roughage it tends to replace the roughage, i.e. the substitution effect is great. The substitution effect is also greater in beginning of lactation. (Harstad, 1994, Kokkonen et al., 2004). Dann et al., (1999) found no effect on prepartum or postpartum dry matter intake when feeding differently available carbohydrates but energy intake was greater for cows fed more available carbohydrates. Kokkonen et al., (2004) also found no significant differences in DMI postpartum between low, medium and high concentrated diets prepartum.

1.4 Body weight and condition score

Many factors affect body weight including lactation status and feeding. Approximately 20% of cow weight is the content of the rumen and the intestines. In the last 5 months of the pregnancy the weight of the fetus starts to influence the total weight of the cow and at the end of the pregnancy, fetal fluid, fetal membranes and the calf is considered to weigh 15% of the total weight of the cow. Milking and drinking water has also a great effect on the weight, that's why it is important to weigh cows at the same time of the day, considering milking and feeding. It is very well known that cow's body condition has a great effect on milk yield and health. Cows build up body reserves before calving to use in support of lactation (Ingvartsen, 2006). Cows in good condition have a tendency to milk more than cows that score lower (Stockdale, 2006). Fat cows are in more risk of metabolic imbalance and production related diseases than cows in moderate condition. To ensure high production and healthy cows it is important to keep an eye on their condition. The ideal body condition for cows during each stage of lactation is the condition that optimizes milk production, minimizes health and reproductive disorders, and maximizes economic income (Gearhart et al., 1990).

Before calving the cow uses the diet for maintenance and fetal development, after calving she starts to produce milk and the energy supply from the diet is not sufficient. The cow starts mobilizing fat from her body before calving to use as an energy source. The same thing applies for specific nutrients, when milk production starts the need for glucose, protein and fat is large and because of less feed intake the cow needs to use body reserves. If however fat mobilization is extensive, fat starts to accumulate in the liver. Douglas et al., (2006) found that cows fed restrictive diets lost less BW 3 weeks prepartum than cows fed *ad libitum* prepartum. Research has shown that thin cows maintain body condition before parturition while fat cows lose condition (Stockdale, 2006) and rapid loss of body condition can be associated with a higher incidence of metabolic disorders and fertility problems (Gearhart et al., 1990). It is recommended to keep the body condition score (BCS) at 3,0 - 3,5 (Bjarnadóttir, 2001, Gillund et al., 2001). Gearhart et al., (1990) found that ideally BCS at drying off should be the same as desired at calving, because loosing condition in the dry period increases the odds of health problems. Gillund et al., (2001) found that cows at calving with a BCS as high or higher than 3.5 were approximately 2.5 times more likely to become ketotic than cows with scores 3.25 or lower.

1.5 Milk yield and composition

There is a steady loss of epithelial cells from the alveoli during lactation and when the cow has passed peak lactation, more cells are lost than are formed. When a lactating cow becomes pregnant, the increase in concentration of estrogen and progesterone will reduce milk production more than if not in calf. It is possible to maintain milk production until calving but formation of new epithelial cells, and therefore milk production during the next lactation, will be lower than when cows are given a break from milk production (Sjaastad et al., 2003). Milk production will decrease if the udder is only partially emptied for a period of time, because some alveoli and secretory tissue will undergo involution (Sjaastad et al., 2003).

The components of milk are primarily synthesized from nutrients absorbed from the digestive tract, but triglycerides and amino acids mobilized from the body can also be utilized (Sjaastad et al., 2003). Mammary epithelial cells use glucose from blood for synthesis of the disaccharide lactose, which is found only in milk. Glucose is an important energy source for monogastric animals but in the ruminant almost all glucose from the diet is used by the bacteria in the rumen, very little is absorbed. That is why ruminants need to produce glucose from other sources. The most important substrates used in gluconeogenesis are propionic acid, amino acids and lactate. That is why it is important that the diet supplies enough propionic acid and amino acids for absorption. In case of plasma glucose deficiency, secretion of lactose will fall and milk volume will be greatly reduced. Uptake of glucose by the mammary gland increases to a great extent in lactation, while uptake and utilization of glucose in muscles and adipose tissue is reduced. High milk production leads to a drop in glucose concentration in blood. This fall in glucose concentration reduces insulin secretion which further reduces the uptake of glucose by muscle and adipose tissue. Competition for available glucose is thereby reduced and the udder can continue its high utilization of glucose, a prioritized organ (Sjaastad et al., 2003).

During fatty acid synthesis, both long-chain fatty acids (C16 and C18) and fatty acids with shorter carbon chains (C4 to C14) are formed. The short-chain fatty acids characterize milk fat. Around 40-50% of fatty acids in milk are produced in the mammary epithelial cells by synthesis from smaller components, such as acetate. The other half, the preformed fatty acids, are taken up from the blood. Amino acids are taken up from the blood for synthesis

of milk proteins, casein is the main protein and is specific for milk (Sjaastad et al., 2003). Milk production is primarily determined by genetic potential for milk production, by feeding around puberty and in the dry period close to parturition, and by the ability of feed consumed to supply the substrates required for milk synthesis (Sjaastad et al., 2003).

Protein concentration in milk is affected by amino acids absorbed from the intestines; reduced amino acid absorption will reduce protein concentration in the milk. Feeding additional protein prepartum, particularly protein that is not degraded in the rumen, minimizes the use of body protein reserves for the fetus. These reserves are then available to support milk protein production (Moorby et al., 1996). Doepel et al., (2002) points out that milk synthesis will occur if protein is available even when energy is limiting, and cows that have excessive mobilizable protein reserves are promoted to greater lipid mobilization to meet energy requirements. Kokkonen et al., (2004) adds that a diet of inadequate energy density may force cows with high milk yield potential to mobilize excessive amounts of lipids and thus increasing the risk of ketosis and other health problems. Milk urea N has been used as indicator of protein utilization. If urea concentration in milk is abnormally low, the feed is usually deficient in nitrogen and the animal must then mobilize body protein to produce milk.

Dairy cow feeding postpartum should meet all nutrient requirements as well as possible so the cow produces to its maximal potential without risking good health. Increased milk production can result from one or a combination of the following; increased microbial protein synthesis, increased propionate concentration, increased postrumenal digestion of starch and increased bypass protein (Dann et al., 1999).

1.6 Fertility

When a female is in estrus she tends to be restless and seeking a mate. If she becomes pregnant, the next estrus is postponed so the embryo has time to develop. If a female in estrus does not become pregnant new follicles start to develop on the ovaries in preparation for a new estrus (Sjaastad et al., 2003). Length of the estrus cycle is 21 days in cows. Corpus luteum which is formed in the ovaries after ovulation is responsible for the production of progesterone. Secretion of progesterone starts when corpora lutea starts to develop the ovaries in the luteal phase of estrus. If the cow becomes pregnant corpora lutea

continues to secret progesterone but progesterone secretion drops when the corpora lutea undergoes involution if the cow does not get pregnant (Sjaastad et al., 2003). Because of increased progesterone concentration the uterus undergoes changes that will increase acceptance and nourishing of the embryo. If the cow becomes pregnant the most important effects of progesterone is to create favorable conditions in the uterus for the embryo to develop. Progesterone prevents new ovulation, it stimulates growth and development of the uterus and it also contributes to growth and preparation of the mammary gland for milk synthesis (Sjaastad et al., 2003). Progesterone in plasma and in milk can help diagnosing pregnancy. Animals that have a low progesterone concentration are not pregnant. But after finding high concentration of progesterone in a single sample we cannot be sure of pregnancy and repeated measures are needed. If a sample is taken 19-21 days after insemination from pregnant cows, progesterone concentration is high. Non-pregnant cows would at that time be in the follicular phase and not producing progesterone (Sjaastad et al., 2003).

A healthy and safe passage through the transition period is crucial for proper health, welfare, production and reproductive efficiency of the cow (Roche et al., 2000). It has been suggested that mobilization after calving and timing of changes may be extremely important for reproductive performance (Stockdale, 2006). Most cows undergo negative energy balance in the first weeks of lactation and thereby a low level of insulin. Insulin induces receptors for the hormone LH in the granulose cells and thereby modulates the ovulation rate (Kruip et al., 1998). That is why negative energy balance influences fertility. Supporting this, it has been hypothesized that decrease in fertility of dairy cows is related to increase in milk yield and associated with fatty liver and other metabolic disorders (Gillund et al., 2001, Butler et al., 1981).

1.7 Liver condition

The liver handles among other things most of the nutrients absorbed from the intestine and regulates their release into the blood. Fat mobilization in the event of negative energy balance, often occurs around calving. Then fat is mobilized from tissue to the liver. Factors stimulating mobilization of free fatty acids are as mentioned, over conditioned cows, high yield, and diets low in energy. If the mobilization is too extensive, lipids and triglycerides accumulate in the liver (Bremmer et al., 2000, Bertics et al., 1992) which makes it harder

for the liver to deal with increased chemical imbalance in the beginning of lactation, with the consequences of increased risk of ketosis and other production diseases (Harðarson, 2003). NEFA (non-esterified fatty acids) concentration in blood is a good indicator of liver condition because of a positive correlation between blood NEFA concentration and fat accumulation in liver (Harðarson, 2003). When NEFA have been taken up by the liver they can be oxidized to CO_2 to provide energy for the liver, partially oxidized to produce ketone bodies that serve as energy sources for other tissues, or reconverted to triglycerides (Ingvartsen, 2006, Harstad, 1994, Harðarson and Ingvartsen, 2005). Triglycerides are exported from the liver within very low density lipoproteins (VLDL) that are synthesized and secreted at a low rate by ruminants, which thereby favors triglyseride (TG) accumulation in cells and development of fatty liver (Drackley et al., 2006). This is supported by findings of Bertics et al., (1992) who found that liver total lipid tended to be higher and liver TG was significantly higher in cows that experienced a reduction in feed intake prepartum, and thereby increased mobilization of fat. They also found that cows that were force fed had an increase in TG concentration which may indicate that other factors than feed intake contribute to fatty liver development, such as insulin, estrogen and other hormones elevated at calving (Bertics et al., 1992).

Fatty liver syndrome is most often a sub-clinical condition, but when it occurs clinically the symptoms are depression, lack of appetite and weight loss, most cows will suffer from non-specific clinical symptoms as reduced rumen activity and decreased milk yield (Ingvartsen, 2006). Reid and Roberts, (1982) gave the classification that cows with less than 20% fat in the liver one week after parturition are considered normal and cows with more than 20% fat in the liver are considered to have fatty liver. Based on that classification they found that roughly 1 in 3 cows have fatty liver one week after calving. Fatty liver can also be a secondary disease of other production diseases that depress appetite and increase mobilization of body fat (Ingvartsen, 2006). Fatty liver has been related to increased rate of other production diseases such as mastitis, displaced abomasums, retained placenta, ketosis, and also to increased production losses (Bremmer et al., 2000). Fatty liver does not necessarily involve severe liver dysfunction and the liver can return to normal structure and function once the metabolic status is corrected (Jubb et al 1993; seen in (Harðarson and Ingvartsen, 2005).

1.8 Blood parameters

Blood parameters can be used as indicators for metabolic status of the cow, blood parameters represent what is going on in the body. These parameters are, for example: non-esterified fatty acids (NEFA), glucose, insulin, growth hormone, the ketone bodies; acetoacetate, β-hydroxybutyrate and acetone in blood and liver enzymes such as AST, GGT, GLDH and ICDH.

The ratio of growth hormone to insulin in blood is high in cows in early lactation, stimulating mobilization of long-chain fatty acids from adipose tissue to support lactation demands. These fatty-acids circulate as non-esterified fatty acids (NEFA) in blood and are a major source of energy to the cow during this critical period (Ingvartsen, 2006). Cows fed high energy diets will have greater insulin resistance which will result in higher NEFA plasma concentration (Holtenius et al., 2003). Bertics et al., (1992) suggest that increasing blood glucose may elicit an insulin response and reduce fatty acid mobilization from adipose tissue. When DMI increases after calving NEFA concentrations decline (Doepel et al., 2002). NEFA concentration in blood can reflect fat mobilization in the periparturient period. Plasma NEFA is a useful parameter to help evaluate energy balance of cows, because they are elevated when stored fat is mobilized (Holcomb et al., 2001). Fat mobilization usually starts in the end of the dry period and reaches a peak in early lactation (Ingvartsen, 2006). Decreased DMI in the dry period will result in poor energy balance and therefore in an increased NEFA concentration in blood after calving (de Boer et al., 1985, Drackley, 1999, Ingvartsen, 2006, Bremmer et al., 2000, Doepel et al., 2002), which is probably a direct result of the decrease in plasma insulin concentration (Bremmer et al., 2000). Research have shown that when cows are fed more energy in the dry period than is required insulin concentration in blood is higher than in cows fed less energy than required (Dann et al., 2006, Holtenius et al., 2003). Research does not always show the same results in feeding high concentrated diet prepartum, some have found that feeding high energy diets prepartum has a positive effect on NEFA concentration in plasma (Doepel et al., 2002, Holcomb et al., 2001) and then there are others that have found no significant effect on plasma NEFA concentration with different energy allowance (Holtenius et al., 2003). NEFA concentration in blood is high first after parturition while glucose concentration is reduced. NEFA concentration starts to rise 2 to 3 weeks before calving, around parturition or during the first week of lactation. Glucose concentration usually increases during the

last week prepartum, drops quickly postpartum and reaches a minimum 1 to 3 weeks in lactation (Ingvartsen and Andersen, 2000). These changes reflect the large need for glucose and other nutrients by the mammary gland and also that dairy cows use lipid in an increased measure as a source of energy in lactation (Ingvartsen and Andersen, 2000).

When there is a glucose deficiency, lipid mobilization is stimulated resulting in increased ketogenesis, and thus high concentrations of ketone bodies in blood, milk and urine (Ingvartsen, 2006). The metabolic disease Ketosis occurs when concentration of the ketone bodies; acetoacetate, β-hydroxybutyrate and acetone are high and glucose concentration is low in blood. Postpartum changes in the plasma concentration of β-hydroxybutyrate (BHB) are generally opposite to those of glucose. Ketone bodies are produced by ketogenesis and have the primary function to transform excessive fatty acid carbon in the liver to an easily oxidizable form for tissues to use instead of glucose (Ingvartsen, 2006). The ketone bodies accumulate in the blood because there is a lack of oxalacetate, it is therefore not possible to oxidize all the ketone bodies that come from the liver (Harstad, 1994). This is most often seen in the first month of lactation, and less frequently later on in lactation (Ingvartsen, 2006, Harstad, 1994). Health records available are very limited for the Icelandic cows, but according to them the Icelandic cow is sensitive for production diseases and the incidence rate of clinical ketosis is around 20% (Harðarson and Ingvartsen, 2005).

Symptoms for ketosis are well known, such as; reduced eating, decline in milk yield, hard, dark slimy manure and acetone smell from breath, urine and milk (Harstad, 1994). Ketosis is often categorized, but not all use the same categories. Most generally there are two categories, primary ketosis and secondary ketosis (Harstad, 1994). When a primary ketosis develops the main reason is that too little energy is taken up in feed, which can come from; too little feed available, diet with bad quality, unsuitable feeding. Forage structured diets that give little material for gluconeogenesis or diets that are high in butyric acid can also generate primary ketosis (Harstad, 1994). Doepel et al., (2002) found that feeding diet high in protein prepartum gave significantly higher BHB concentrations than feeding diet low in protein. Butyric acid is generated to β-hydroxybuterate in the rumen which increases the amount of ketone bodies available and increases the danger for ketosis to develop (Harstad, 1994).

Secondary ketosis develops as a result of negative energy balance that results from other disorders such as, displaced abomasum, chronic rumen acidosis, which will reduce glucose concentration in blood and increase NEFA and ketone bodies concentration in blood (Ingvartsen, 2006, Harstad, 1994).

2. Aims of study

The main objective of this study was to estimate the effect of three diets of different energy levels fed prepartum on dry matter intake, performance and health of Icelandic dairy cows.

In Manuscript I results regarding the effects of the three dietary treatments upon feed intake, live weight, body condition, milk yield and composition and fertility are covered.

In Manuscript II results regarding the effects of the three dietary treatments upon liver condition and blood parameters are covered. Fat content in liver cells was estimated by microscopical methods and the metabolic status of the body around parturition was evaluated by measuring glucose, BHB, NEFA and liver enzymes in blood.

The thesis combines the result from Manuscript I and Manuscript II and covers how all these parameters are connected.

3. Materials and methods

The experiment was performed at the research farm Stóra- Ármót, using 30 Icelandic cows and heifers in individual tie stalls. The animals were blocked by parity (1, 2 and 3+) and randomly assigned with one of three dietary treatments. Complete feeding with equipment from Mullerup A/S Denmark was used; the system was programmed for mixing the diets and feeding the cows. Feeding was *ad libitum*, refusals were weighed three days a week and feeding was adjusted for 5-10% refusals twice a week. Water was available at all times.

3.1 Diet composition

Six diet compositions were used in the experiment. Diets were composed of hay, straw, barley and compounded feed in different ratios (Table 2). In the first period, 8-4 week's prepartum, all cows were fed the same diet. In the second period, 3-0 week's prepartum, cows were on three different dietary treatments. In the third period, 0-3 weeks postpartum, all cows were fed a diet with a composition formulated for fresh cows. In period four, 4-12 weeks postpartum, all cows were fed a diet with a composition formulated for high yielding cows.

Table 2. Composition of diets.

Weeks	Treatment	Feed			
		Hay	Straw	Barley	Concentrate
8-4	All	60%	40%	-	-
3-0	DL	60%	35%	-	5%
3-0	DM	60%	20%	-	20%
3-0	DH	65%	-	-	35%
0-3	All	54%	-	7%	39%
4-12	All	38%	-	25%	37%

Samples of the forage were taken once a week and samples of barley and compounded feed were taken every two weeks, to be analyzed for: dry matter, crude protein (CP) using Kjeldahl method, Neutral detergent fiber (NDF) using ANKOM technology on Van Soest method (Van Soest et al., 1991), ashes, dry matter digestion (DMD) using a modified Tilley and Terry method (Tilley and Terry, 1963) and minerals. Dry matter was determined twice a week for the forage but once a week for the barley and from the refusals of every diet. Table 3 shows the chemical composition of every feed ingredient. And Table 4 shows calculated composition of the diets.

Table 3. Dry matter (DM) content in % of wet weight and chemical analysis of the feed, in % of DM.

Feed	DM	CP	NDF	Ash	DMD	FE_m [1)]	Ca	P	Mg	K	Na
Hay	30,85	20,21	46,20	9,02	72,81	0,84	0,45	0,40	0,21	2,21	0,09
Straw	79,39	7,27	72,16	6,25	44,0	0,44	0,23	0,11	0,89	0,11	0,12
Barley	49,09	12,50	15,29	2,90		1,14	0,04	0,30	0,11	0,63	0,02
Compounds	86,82	20,46	21,79	9,03		1,12	1,44	1,14	0,83	1,04	0,42
Prepartum Compounds postpartum	88,05	22,28	179,3	9,08		1,12	2,03	1,15	0,49	1,01	0,48

[1)]Calculated energy value (FE_m) is per kg DM.

Table 4 Composition of diets.

	8-4	3-0 DL	3-0 DM	3-0 DH	0-3	4-12
DM %	50,27	50,64	51,75	50,44	54,43	56,57
CP	15,03	15,69	17,67	20,30	20,48	19,05
NDF	56,58	54,07	46,51	37,66	33,01	28,01
FEm	0,68	0,71	0,82	0,94	0,97	1,02

3.2 Weighing and condition scoring

The cows were weighed and condition scored once a week from week 8 before calving until 12 weeks after calving. Cows were weighed after the morning milking, before feeding. Condition scoring was based on a scale from 1-5 where 1 is very thin and 5 very fat. Five spots on the cow were used to estimate the condition; sacrum, lower part of the ribs, wing of ilium, caudal vertebra and ischium. In an article by (Bjarnadóttir, 2001) the method and scale for condition scoring Icelandic cows is illustrated.

3.3 Milk sampling

Cows were milked 2 times a day at 07:00 and 18:00. Milk yield was registered automatically by a SAC milking system at every milking. Energy corrected milk was calculated according to (Sjaunja et al., 1990). Every week, at one morning and one evening milking, milk samples were taken and sent to the research center for the milking industry in Iceland (Rannsóknarstofa mjólkuriðnaðarins) and analyzed for fat, protein, urea, lactose, free fatty acids, casein and somatic cell count. The concentration of progesterone was estimated in milk three times a week, Monday, Wednesday and Friday, using a manual kit from Ridgeway Science in England, starting 14 days after calving. Progesterone in milk samples was measured by a method based on enzymatic degradation of progesterone.

3.4 Liver sampling

For assessing the condition of the liver 23 needle biopsies were taken from the liver of 22 cows around calving. Samples were taken in the period from 3 days prepartum to 8 days postpartum, using puncture biopsies (Hughes 1962). The area between the 11th and 12th ribs on the right side was clipped and disinfected. Then the area was anaesthetized by using a local anaesthetic lidocain. Small incision was made parallel to the ribs and the needle directed towards the liver. The samples were at first stored in liquid nitrogen and then fixed in 10% buffered formalin. After that they were embedded in paraffin, sectioned at 3 µm thickness and stained with hematoxylin and eosin. Cellular fat content was rated using two methods: firstly, manually under a microscope where samples were rated from 0 to 5 (Steen et al. 1992), 0 being normal liver cells and 5 almost all cells having filled vacuole cytoplasm and pyknotic nucleus; and secondly by using the computer program Leica QWin. The computer program calculated total area of fat in sample, mean area of fat in sample and number of fat features in sample.

3.5 Blood sampling

Blood sampling was made once a week, from 4 weeks before expected calving until 6 weeks after calving and then 8, 10 and 12 weeks after calving. Blood was drawn from the coccygeal vessel under the tail into a lithium heparin tube, to prevent coagulation. The tubes were put in ice-bath to stop metabolic reactions. After centrifugation plasma samples were put into a freezer and stored at -20°C until they were analyzed for glucose, BHB, NEFA, AST, GGT, GLDH, and ICDH. Blood plasma glucose, AST, and GGT were determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650). NEFA were determined using the Wako, NEFA C ACS-ACOD assay method. BHB was determined as an increase in absorbance at 340 nm due to the production of NADH, at slightly alkaline pH in the presence of β-OH-butyrate dehydrogenase. Sample blank was included. The method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by (Harano et al. 1985). GLDH activity was quantified in a kinetic, colorimetric assay according to (Schmidt&Schmidt 1995). ICDH was determined in a kinetic, colorimetric assay using isocitrate as substrate and $NADPH_2$ as response parameter. All analyses were performed using an autoanalyzer, ADVIA 1650 ® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA). Precision of all parameters was below 2 CV% (intra assay) and 4 CV% (inter

assay). Chemicals analyzed from blood plasma are listed in table 3 and a short description of their usage is given.

3.6 Ketolac® test

Ketolac stick test (Sanwa Kagaku Kenkyusho co.Ltd., Japan) was used to detect the level of β-hydroxy-butyrate (BHB) in milk. The stick contains β-hydroxy-butyrate-dehydrogenase which converts β-hydroxy-butyrate (BHB) and NAD^+ into acetoacetic acid (ACA) and NADH. At the same time NADH reduces the Nitrotetrazolium-blue (NTB) contained in the stick to the purple Formazan. By comparing the intensity of the colour change on the stick with a colour chart it is possible to determine β-hydroxy-butyrate (BHB) concentration in milk. The test was performed 3 times per cow, 1, 2 and 3 weeks after calving.

3.7 Statistical analysis

Statistical analyses were performed in the computer program MINITAB 14 (Minitab, Inc. Pennsylvania, USA). For analyses of DMI, body weight and condition score, production and fertility, GLM procedure was used to detect effect of treatment, period and parity (Manuscript I). Treatment-period interaction and treatment-parity interaction was also tested on DMI using GLM procedure.

$$Y_{ij} = \mu + \alpha_i + \gamma_j + \alpha\gamma_{ij} + \varepsilon_{ij}$$

Where Y_{ij} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} period, $\alpha\gamma_{ij}$ is the interaction between treatment and period and ε_{ij} is the residual effect. Same model was used to test parity where γ represented parity. DMI in every period separately was tested against treatment to see if there was a significant effect of treatment in individual periods.

Treatment, period and treatment-period interaction was tested on weight and body condition score (BCS). Weight change and BCS change was found by using descriptive statistics.

Treatment, period, parity and all possible interactions between these were tested on ECM, protein, fat, lactose and urea concentration in milk. Ovulation and number of inseminations were all found using descriptive statistics. Effect of treatment was tested on ovulation and insemination frequency using GLM procedure.

Statistical analyses were performed in the computer program MINITAB 15 (Minitab, Inc. Pennsylvania, USA) for analysis of blood parameters, liver parameters and ketolac test (Manuscript II). GLM procedure was used to detect effect of treatment, period and parity on glucose, AST, GGT, BHB, GLDH, ICDH and NEFA. Treatment-period and treatment-parity interactions were also tested.

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \delta_k + \alpha\gamma_{ij} + \alpha\delta_{ik} + \varepsilon_{ijk}$$

Where Y_{ijk} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} period, δ_k is the effect associated with the k_{th} parity, $\alpha\gamma_{ij}$ is the interaction between treatment and period, $\alpha\delta_{ik}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

GLM procedure was also used to determine whether treatment, parity or body condition score (BCS) affected BHB in milk.

$$Y_{ijkl} = \mu + \alpha_i + \gamma_j + \delta_k + \eta_{l+} \alpha\gamma_{ij} + \alpha\delta_{ik}\ \varepsilon_{ijk}$$

Where Y_{ijkl} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} week (1,2,3), δ_k is the effect associated with the k_{th} parity, η_l is the random effect associated with the l_{th} body condition score, $\alpha\gamma_{ij}$ is the interaction between treatment and week, $\alpha\delta_{ik}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

The relationship between BHB measured in milk and BHB measured in blood was tested using Regression procedure.

When estimating the liver condition, GLM procedure was used where treatment and parity were tested against the liver parameters, total area of fat, mean area of fat, number of fat features and fat content rated.

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \delta_k + \alpha\gamma_{ij} + \alpha\delta_{ik}\ \varepsilon_{ijk}$$

Where Y_{ijk} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} parity, $\alpha\gamma_{ij}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

Weight, BCS, ECM and liver enzymes were also tested individually against the liver parameters.

Energy calculations were performed using equations from the co-Nordic feed evaluation system NorFor Plan. Firstly, energy requirements for maintenance, pregnancy, performance and growth were calculated. Energy requirements for each period were then found by adding calculated energy needs for appropriate factors. Then energy contents of the feed were calculated also using NorFor Plan. Netto energy lactation (NEL) was found for every feed stuff. In NorFor Plan DMI affects NEL of the feed, NELp8 and NELp20 were therefore used. NELp8 is the net energy in MJ/DM when 8 kg DM is fed and NELp20 is the net energy in MJ/DM when 20 kg DM is fed. NELp8 was used when calculating energy consumed prepartum but NELp20 was used while calculating energy consumed postpartum because of greater DMI postpartum than prepartum. Energy requirements were calculated separately for heifers and multiparous cows because the heifers need energy for growth but the older cows do not. Energy requirements were also calculated for each period for comparison of different feeding in each period. Energy requirements were also calculated separately for each treatment. The same thing applied for calculations for energy consumed.

4. Results

Results are viewed in more detail in manuscripts I and II, were manuscript I covers results from DMI, lactation performance and fertility and Manuscript II covers results from blood and liver parameters. In this chapter the results will be presented more graphically and connecting the results from both manuscripts in one.

Due to differences between actual and expected calving dates, there was some discrepancy between cows for how long they received the experimental ration. The average time on the close up diets was 4.6 weeks. 20 cows received the experimental ration for 3-4 weeks and only 4 cows received the experimental ration for less than 3 weeks. Average time for cows in treatment 4 to receive the experimental ration was 5.6 weeks, for cows in treatment 5 the average time for receiving the experimental ration was 4.5 weeks and cows in treatment 6 received the experimental ration in 3.9 weeks on average.

4.1 Dry matter intake

DMI was significantly affected by treatment, periods and parity. DMI over the whole experiment was about 16% higher in treatment DH than DL. Dry matter intake in the period 8-4 wk prepartum was not significantly different between treatments; DMI in the period 3-0 wk prepartum was significantly different between treatments as DMI increased with increased energy concentration in the diet. Figure 1 presents DMI in all treatments from 8 weeks prepartum to 12 weeks postpartum. DMI postpartum was not significantly different between treatments. As could be expected, the increase in DMI after calving was greatest for treatment DL and smallest for treatment DH. This was especially apparent for the heifers. Parity affected DMI, i.e. heifers consumed less than older cows. Results from DMI are presented in more detail in manuscript I with tables and p-values.

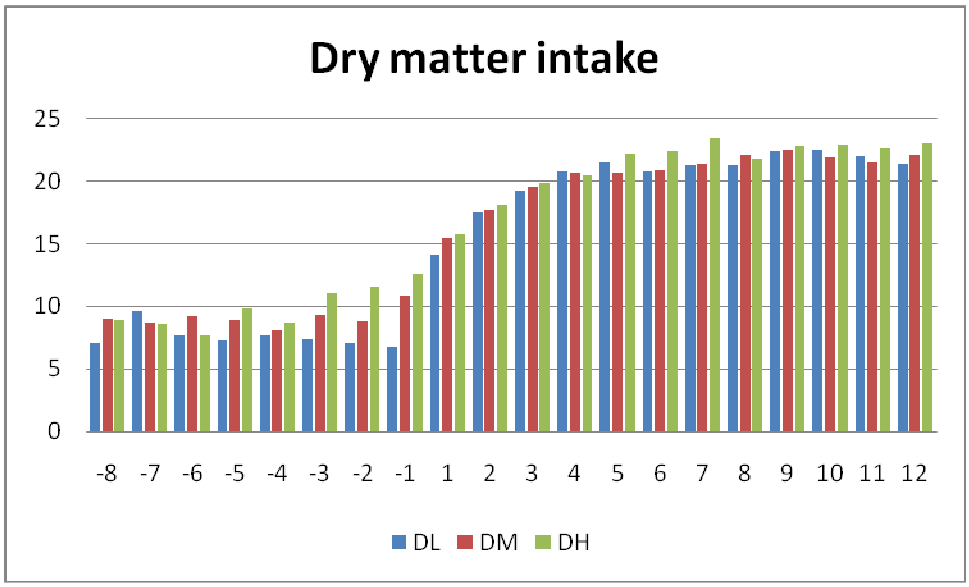

Figure 1. DMI in kg/DM from 8 weeks prepartum to 12 weeks postpartum.

4.2 Body weight and condition score

Body weight and condition score were significantly different between treatments. However, cows were not assigned to treatments by weight so changes in weight and body condition tell more about treatment effect on weight and body condition. Body weight and condition score changes are presented in manuscript I. Treatments did not affect body weight or condition score change significantly. Periods affected body weight and condition score change significantly. The cows gain weight as parturition approaches and then lose weight postpartum, the same thing applies for BCS.

4.3 Milk yield and composition

There was not a significant difference in energy corrected milk (ECM) between treatments. Cows in treatment DL showed on average lower yield than cows in other treatments, but this was not significant as mentioned before. ECM in the different treatments can be viewed in figure 2. There was a significant difference in fat content between treatments and also between periods, cows in treatment DL gave the highest fat in milk and there was higher fat in the first three weeks postpartum than in weeks 4-12. Treatments and periods affected urea concentration significantly, cows in treatment DL had highest urea in milk and there was higher urea in the period of 4-12 weeks postpartum than in the first three weeks of lactation. Parity affected ECM, protein and lactose significantly, heifers had lower ECM and higher protein and lactose than older cows. The differences in ECM, fat,

protein, lactose and urea content between treatments and by parity are presented in manuscript I.

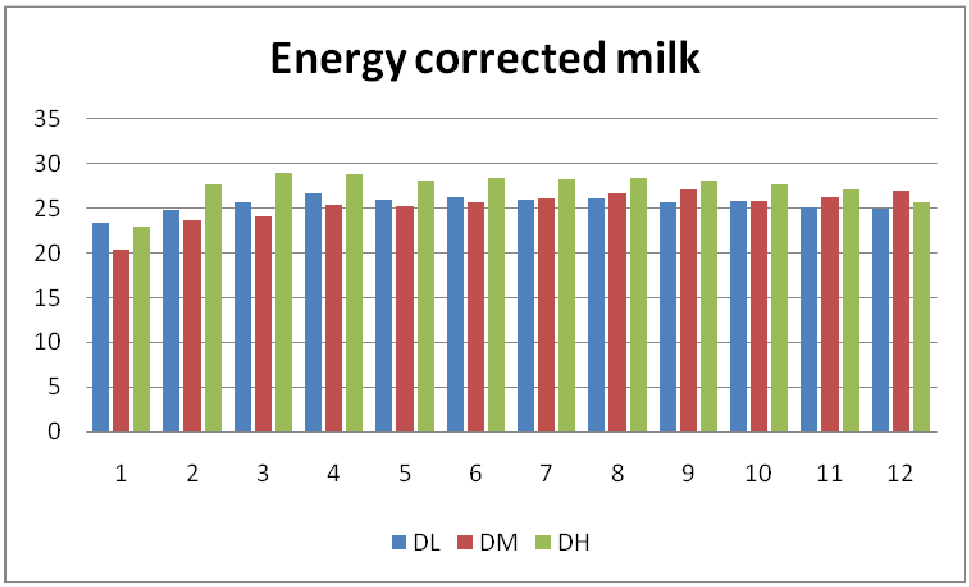

Figure 2. ECM/day from week 1 to week 12 in lactation.

4.4 Fertility

After viewing progesterone concentration in milk, time of first ovulation was determined and only few cows showed regular oestrus in this period. There was not a significant difference in time of first ovulation or number of inseminations needed per conception between treatments. Results from fertility parameters are presented more precise and in a table in manuscript I.

4.5 Liver samples

Treatments did not have a significant effect on total area of fat in sample, mean area of fat in sample or number of fat features as estimated by the Leica Qwin computer program. Treatments did not affect cellular fat rating under a microscope. Parity had a significant effect on total area of fat in sample, mean area of fat in sample and number of fat features found in sample in the computer program. Parity also affected fat rating under a microscope significantly. Liver fat increased with increased number of parturitions. Average body weight in the last three weeks prepartum affected total area of fat, mean area of fat and number of fat features found by the computer program. Weight also affected fat rating under a microscope. Heavier cows had more fat in their liver than lighter cows. Mean body condition score the last three weeks prepartum was significantly correlated to mean area of fat, where cows with higher BCS had more fat in the liver. There was also a

trend for increased number of fat features found with increased BCS. BCS also affected cellular fat rating under a microscope significantly; cows with higher BCS were rated higher. Mean ECM for the first 12 weeks in lactation was correlated to total area of fat, mean area of fat and number of fat features found in the computer program. Cows with higher ECM the first 12 weeks in lactation also had more fat in the liver. The same thing applied for ECM and cellular fat rated under a microscope. Liver enzyme concentration in blood and liver condition was positively correlated. Liver enzyme concentration in blood increased with increased fat in liver. Liver enzyme results are presented in more detail with tables and p-values in manuscript II.

4.6 Blood parameters

There was a significant effect of treatment on plasma glucose concentration where plasma glucose concentration increased with increased concentrates in the diet. Glucose results can also be viewed in manuscript II. There was also a significant effect of periods, parity and an interaction between treatment and period because of lower plasma glucose concentration in period 3-0 wk prepartum for DM cows, but in the other two treatments plasma glucose concentration increased as parturition approached. This increase in plasma glucose was very small for cows in treatment DL. Results from plasma glucose measures can be viewed in manuscript II. Plasma glucose concentration was lowest in the period 0-3 wk postpartum for all treatments, and remained lower in the second postpartum period (4-12 wk) than in the dry period. Glucose concentration for the different treatments from 4 weeks prepartum to 12 weeks postpartum is shown in figure 3. The greatest drop around parturition was for DH cows. Heifers had higher plasma glucose concentration than multiparious cows.

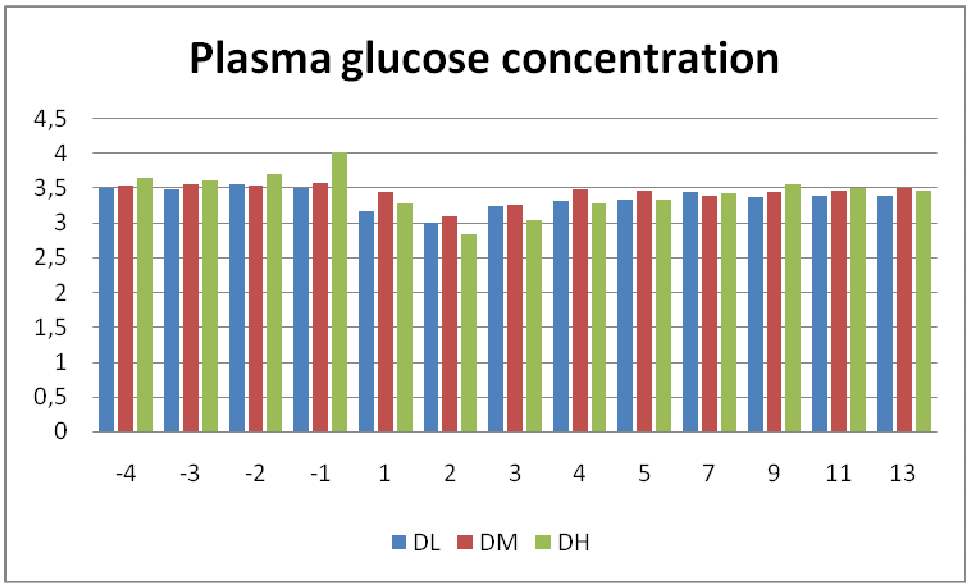

Figure 3. Glucose concentration in blood (mM) from 4 weeks prepartum to 13 weeks postpartum.

There was no significant effect of treatments on plasma BHB concentration, but there was a significant effect of period and parity. Plasma BHB concentration is higher postpartum than prepartum, and by far the highest first after parturition (0-3 wk postpartum). BHB concentration in the different treatments in weeks -4 to 12 are presented in figure 4. Plasma BHB concentration was lower for heifers than for multiparous cows. BHB concentration is presented in tables in manuscript II.

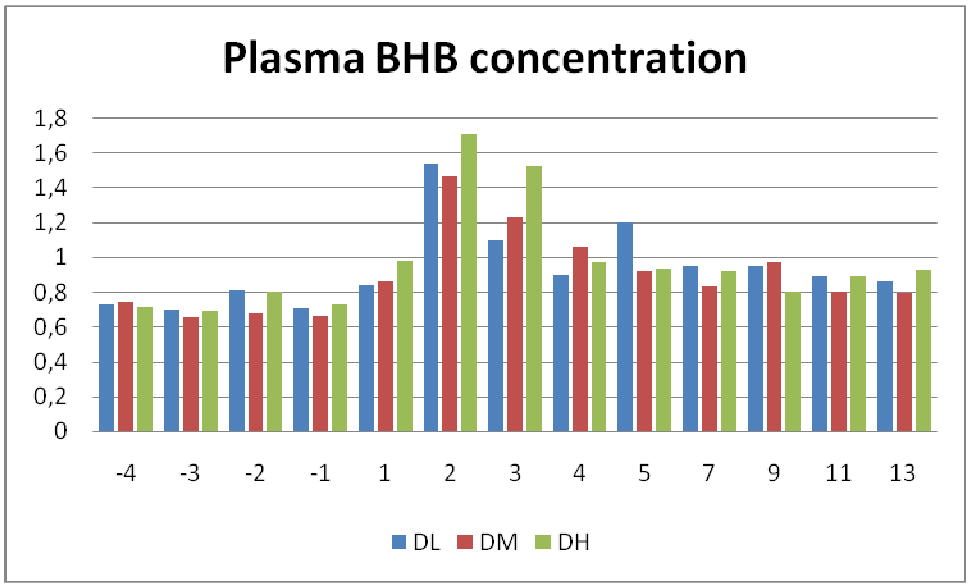

Figure 4. BHB concentration in blood (mM) from 4 weeks prepartum to 13 weeks postpartum.

Treatments did affect plasma NEFA concentration significantly in the period 0-3 weeks postpartum, where cows in treatment DH had the highest NEFA concentration. NEFA concentration can be viewed more precisely in manuscript II. There was a significant effect

of periods. Plasma NEFA concentration was highest in the periods around parturition (3-0 wk prepartum and 0-3 wk postpartum) as can be viewed in figure 5. Plasma NEFA concentration in treatment DL showed lower values on average than the other treatments over all periods, even though it was not significant. There was a trend for plasma NEFA concentration to increase with increased parturitions. There was an interaction between treatment and period. Cows in treatment DM had the highest NEFA concentration 3-0 week's prepartum when cows in DL and DH showed highest NEFA concentration in period 0-3 weeks postpartum.

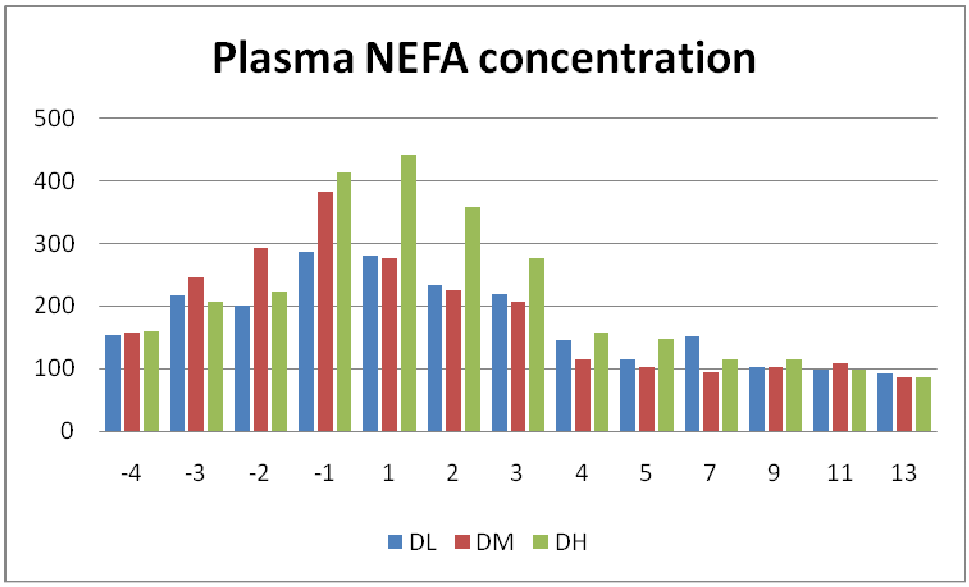

Figure 5. NEFA concentration in blood (µ ekv/l) from 4 weeks prepartum to 13 weeks postpartum.

There was no significant effect of treatments on plasma AST concentration, but there was a significant effect of periods on plasma AST concentration. Plasma AST concentration increased after parturition and remained higher postpartum than in the dry period. Plasma GGT concentration was significantly affected by period, parity and there was an interaction between treatment and parity. Heifers have the lowest plasma GGT concentration and cows in lactation 3+ have higher concentration than cows in their second lactation. Plasma GLDH concentration was significantly affected by treatment and period. Plasma GLDH concentration was positively correlated with concentration in diet, where DL cows had the lowest concentration and DH cows the highest. Plasma GLDH concentration was higher postpartum than prepartum and actually increased from the period 8-4 wk prepartum to the period 4-12 wk postpartum. There was a trend for the heifers to have lower plasma GLDH concentration than multiparous cows. Plasma ICDH concentration was significantly affected by period and parity. Plasma ICDH concentration

was higher postpartum than prepartum and the concentration was positively correlated with parity. Liver enzyme concentration is also presented in tables in manuscript II.

4.7 Ketolac test

There was not a significant difference in BHB concentration in milk between treatments, weeks or parity. There was a significant correlation between BHB concentration measured in milk and BHB concentration measured in blood. Regression equation can be viewed in manuscript II.

4.8 Energy balance

Calculated energy requirements are shown in Table 5. Energy requirements increase as parturition approaches, energy needs are also greater postpartum than prepartum. Heifers have greater energy requirements prepartum than older cows but this is reversed postpartum. Heifers have the additional requirements for growth prepartum and older cows have greater requirements for production postpartum.

Table 5. NEL requirements in MJ/day.

	Heifers				Multiparous cows			
	Weeks prepartum		Weeks postpartum		Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**	**8-4**	**3-0**	**0-3**	**4-12**
DL	42,90	54,29	100,51	107,42	37.63	44.18	115.76	116.52
DM	41,38	48,63	86,66	88,32	39.75	48.92	118.93	137.97
DH	37,77	47,79	84,63	97,86	36.38	46.30	127.18	127.27

Energy consumed can be viewed in Table 6. Energy consumed follows DMI and increases from the first period to the last. Energy consumed is greatest for cows and heifers in treatment DH and lowest in treatment DL in the experimental period. This does not maintain into the lactation.

Table 6. NEL consumed in MJ/day.

	Heifers				Multiparous cows			
	Weeks prepartum		Weeks postpartum		Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**	**8-4**	**3-0**	**0-3**	**4-12**
DL	37,49	40,69	118,57	142,29	52,67	44,15	118,46	161,19
DM	49,86	55,33	102,99	126,80	58,50	65,40	137,95	176,40
DH	48,01	73,24	100,01	126,72	51,34	84,58	135,95	174,14

The difference between calculated energy requirements and calulated energy consumed for all treatments is shown for heifers in figure 6 and cows in figure 7. Heifers in treatment DL

were in negative energy balance prepartum, they did not consume enough energy to fullfill their needs. Heifers in treatments DM seem to consume just about enough to fulfill their needs prepartum and heifers in treatment DH consumed much more energy than their calculated needs were. Heifers in all treatments consume enough energy to fulfill their needs postpartum.

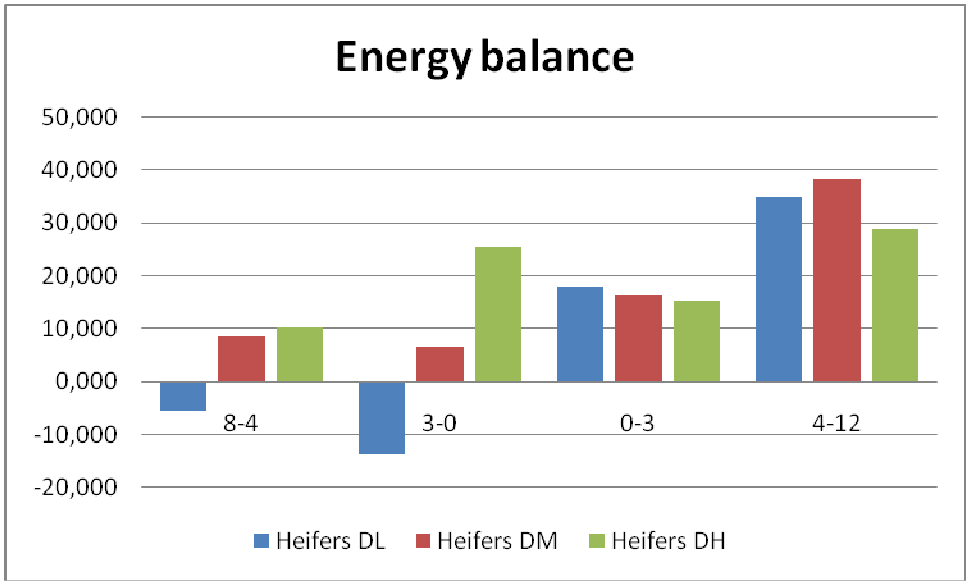

Figure 6. The difference between NEL consumed and NEL needed for Heifers in all treatments in MJ/day.

Cows in treatment DL bearly consume the energy needed in the last three weeks prepartum and the cows in treatment DH consume much more energy than needed the last three weeks prepartum. All cows are consuming enough energy to fulfill their needs postpartum.

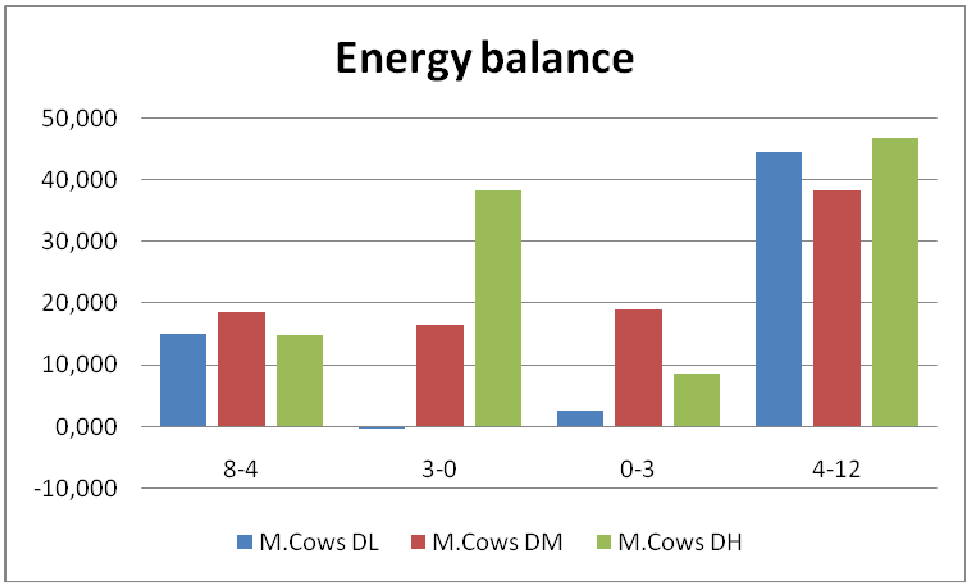

Figure 7. The difference between NEL consumed and NEL needed for multiparous cows in all treatments.

5. Discussion

Dairy cattle feeding in the periparturient period is difficult to manage because of the great energy demands that rise in this period and as lactation commences. Different feeding strategies prepartum to improve postpartum DMI and performance have been the major topic of many trials. It has been suggested that postpartum DMI is positively correlated with prepartum DMI and to improve postpartum performance and health, prepartum DMI should be maximized (Grummer, 1995). In the present experiment, there was no difference in DMI in the period 8-4 week's prepartum, when all the cows were fed the same diet. DMI in the close up dry period was significantly affected by treatments, where DMI increased with increased energy levels in diet. But cows in treatment DH did not sustain increased intake postpartum. This is corresponding to the results of glucose concentration in blood. Glucose concentration in blood plasma increased significantly prepartum with increased energy levels in feed which is also consistent with the results of Rabelo et al., (2005) and cows in treatment DH suffered from greatest reduction in glucose concentration postpartum.

The results from the current study show that cows in treatment DH had higher DMI prepartum than cows in the other two treatments. Therefore they also have a much greater energy consumed than cows in the other treatments prepartum. As parturition approaches energy requirements increase because of a growing fetus and tissues such as the uterus and the mammary gland (Vandehaar et al., 1999), at the same time feed intake often decreases. By increasing the energy density of the diet prepartum increased energy demands as parturition approaches can better by fulfilled (McNamara et al., 2003). Cows in treatment DH were fed diets low in fiber compared to the other two treatments. Diets low in fiber give higher ruminal dry matter digestibility which allows faster rumen digesta evacuation and thereby higher DMI (Rabelo et al., 2003) and can therefore be advantageous considering the decrease in feed intake that normally occurs as parturition approaches.

Cows in treatment DL were fed diets low in concentrate prepartum and they increased DMI postpartum more than cows in the other treatments, and were therefore experiencing much higher difference in starch or easily degraded carbohydrate content than the other two groups between these periods. Including concentrates in the prepartum diet should adapt the rumen microflora to high starch diet postpartum and stimulate the growth of

rumen papillae (Vandehaar et al., 1999; Andersen et al., 1999). Cows in treatment DL may therefore have experienced degeneration in the rumen epithelium and reduction in VFA absorption capacity prepartum. Urea concentration in milk was significantly affected by treatments and periods, where urea concentration increases with decreased energy in prepartum diet. Kokkonen et al., (2004) found that urea in milk tended to be lower with lower energy feeding in the dry period, but was significantly higher when concentrate levels were increased quickly after parturition. This could explain why cows on the DL treatment which had the greatest increase in concentrate levels postpartum, had the highest urea concentration in milk.

Theoretically, grain supplementation during the late dry period will prepare the cow and her rumen for the consumption of high energy concentrated diet when lactation commences, but it is unclear to which extent the feeding in late gestation is likely to prepare the rumen for diets after calving (Stockdale, 2005, Ingvartsen, 2006). According to this, cows in treatment DH and possibly in treatment DM, were better prepared for high concentrate feeding postpartum. Even though their rumen was possibly better prepared for the diets postpartum cows in treatment DH did not increase their DMI as much as cows in the other treatments. They also show the greatest drop in glucose concentration postpartum and the highest BHB and NEFA concentrations in the first three weeks postpartum. This indicates that they had to mobilize more fat from body recourses than cows in other treatments. Vandehaar et al., (1999) suggests that overfeeding carbohydrate in a period prepartum will increase the incidence of hepatic lipidosis which then decreases the glucogenic capacity of hepatocytes and predisposes cows to postpartum metabolic diseases. Cows in treatment DH also showed the greatest body weight drop around parturition. As parturition approaches the body starts sending out hormonal signals that elicit nutrient mobilization form body reserves (Ingvartsen, 2006). This happens because of the dramatic increase in nutrient demand in the beginning of lactation while DMI lags behind. It is therefore a well known fact that BHB and NEFA concentration in blood increases around parturition and is quite normal. Insulin sensitivity in adipose tissue decreases and the release of growth hormone is induced, which increases lipolysis and NEFA mobilization (Dann et al., 1999, Kruip et al., 1998). It can however been used as an indicator of fatty liver when NEFA plasma concentration does not decline shortly after parturition. NEFA results from this experiment are consistent with the results of Vandehaar et al., (1999) were prepartum dietary treatment did not alter plasma NEFA, but there was a

trend for postpartum plasma NEFA to be greater in cattle fed the high energy diet before calving than those fed the low energy diet. Cows in treatment DH showed the highest NEFA concentration around parturition and NEFA concentration in blood postpartum did not decline as fast after parturition as for cows in the other treatments. When lipid mobilization from adipose tissue is severe, triglycerides start to accumulate in the liver and fatty liver develops. Feeding three differently concentrated diets prepartum did not affect fat content in liver significantly. Fat content in milk was significantly affected by treatment where DL cows gave more fat in milk than cows in the other treatments. This is corresponding to the findings of others (Keady et al., 2001; Holcomb et al., 2001). It has been speculated that severe fat mobilization in early lactation increases fat content in milk (Agenas et al., 2003; Keady et al., 2001). Cows with high BCS tend to lose more condition and therefore mobilize more fat. It is therefore possible to speculate that cows in treatment DL were mobilizing more fat from reserves than cows in other treatments.

Our results showed significant increase in plasma glucose concentration prepartum with increased concentrate in feed which is consistent with the results of Rabelo et al., (2005) who found increased glucose concentration in blood when fed high energy diet compared to low energy diet. Research have shown that postpartum glucose concentration is unaffected by different energy concentration prepartum (Douglas et al., 2006, Doepel et al., 2002, Rabelo et al., 2005). This was also that case in this experiment were cows in treatment DH undergo the greatest drop in glucose concentration around parturition and show lowest concentration of glucose in blood the first weeks postpartum after having the highest glucose concentration prepartum. It is normal to see plasma glucose concentration drop postpartum, it is equivalent to higher energy requirements in this period while DMI does not follow. The glucose concentration then increases again when energy metabolism has stabilized few weeks in lactation. BHB concentration gives a better long term indication of feeding than glucose concentration which changes quickly when feeding is changed. BHB concentration postpartum in this experiment was not significantly different between treatments, but cows in treatment DH showed highest concentration and cows in treatment DL showed the lowest concentration. Cows with blood BHB concentration over 1.2 mM were defined as having subclinical ketosis in a study by Enjalbert et al., (2001) and Geishauser et al., (2000) found that cows with more than 1.4 mM of BHB in blood serum had a significantly higher risk of developing clinical ketosis than cows with less than 1.4 mM of BHB in blood serum. Cows in this study showed 1.1 - 1.4 mM BHB in

blood in the period of three weeks postpartum, five cows in this experiment showed light signs of ketosis and they were from all treatments. But cows in treatment DH had the tendency to show higher BHB concentration than cows in other treatments.

Parity also influences DMI, multiparous cows consume more compared with primiparous cows which compares to others (Keady et al., 2001, Rabelo et al., 2003). Heifers in treatment DL consumed about 95% of what the older cows consumed, but the difference was greater in treatments DM and DH where heifers only consumed about 80% of what older cows consumed. This difference can possibly be explained by heifers in treatment DL increasing DMI considerably more postpartum than heifers in the other two treatments. Ingvartsen and Andersen, (2000) point out that in the first part of lactation intake capacity of heifers is only around 80% of that of older cows. The difference in DMI between heifers and older cows decreases as parturition approaches which compares to the findings of Rabelo et al., (2003). The difference in calculated energy requirements and calculated energy consumed was less for heifers than older cow's prepartum. This can be explained by their additional needs for growth. Younger cows have a significantly higher glucose concentration in blood plasma. That can be explained by less production and thereby a lower energy deficiency, DMI can better support their production. Other research has shown the same results (Rabelo et al., 2005). Parity affected liver fat content significantly, i.e. liver fat increased with increased number of parturitions. There was also a trend for increased NEFA concentration with older cows over the whole period. Heifers showed higher NEFA concentration prepartum than older cows. This is corresponding to their lower energy balance in prepartum periods. NEFA concentration in heifers started to decrease sooner after parturition and decreased much faster after parturition than in older cows. This is corresponding to the findings of others (Vandehaar et al., 1999) and can possibly be explained by the fact that heifers produce less milk than older cows which makes it easier to maintain positive energy balance postpartum, or at least minimize the effects of a negative energy balance. Rabelo et al., (2005) pointed out that multiparous cows are more likely to be over conditioned than heifers and are therefore predisposed fatty acid mobilization from adipose tissue which contributes to a greater TG accumulation in liver postpartum.

6. Conclusions

In the present study, DMI in the last weeks prepartum increased with higher energy levels in feed, but this was not significantly maintained into the lactation. This indicates that raising the energy level in prepartum diet could be advantageous for DMI. Because of the concentrated diets prepartum, cows in treatment DH and possibly in treatment DM were better prepared for high concentrate feeding postpartum, which could explain higher DMI postpartum in these groups, even though this increase in DMI was not significant. Cows in treatment DH had a tendency to higher ECM on average than the other treatments, indicating a positive influence of treatment DH on cow performance postpartum even though this was not significant. Cows in treatment DM tended to show a more flat lactation curve than the other treatments, which is beneficial for the cows health. Heifers had significantly less liver fat than older cows, which could be expected. Due to lower production level they can more easily support their production and maintain better metabolic status. Even though few liver and blood parameters were significantly affected by treatments the results from this experiment indicate that cows in treatment DH were in greater danger of developing production diseases than cows in the other treatments. It cannot be recommended to feed as high energy levels as in treatment DH the last three weeks prepartum based on these results. This is concluded after taking into consideration the cost of feeding such high energy when increases in DMI and production are not secure and there is an increased risk of production diseases. Cows in treatment DL were most likely mobilizing more fat from recourses than cows in other treatments which is not beneficial for their health. But cows in treatment DM gave over all the best results, they tended to maintain DMI best postpartum and they did not seem to be in as much danger of developing production diseases around parturition based on blood and liver parameters.

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Effect of dry period nutrition on, dry matter intake, lactation performance and fertility in dairy cattle.

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ABSTRACT

Effect of feed with different energy levels in the close-up dry period (3-0 weeks prepartum) on dry matter intake (DMI), lactation performance and ovarian activity was examined in 30 Icelandic cows and heifers in individual tie stalls. DMI was recorded, live weight and body condition was monitored, milk yield was registered, milk composition analyzed and concentration of the hormone progesterone was measured in milk. DMI prepartum increased significantly with higher energy levels. There were no significant treatment effects on DMI postpartum. Cow weight change and Energy corrected milk (ECM) was not significantly different between treatments. Fat and urea concentration in milk was affected by treatment. Parity had the greatest effects on ECM, protein and lactose. DMI prepartum was higher for cows receiving higher energy levels in feed but they did not maintain significantly higher DMI into the lactation, increased energy prepartum gave higher fat- and lower urea concentration in milk and treatments did not seem to affect fertility.

Keywords: periparturient period, dry matter intake, lactation performance, ovarian activity.

Yfirlit

Markmið rannsóknarinnar var að skoða áhrif mismunandi fóðrunar seinustu þrjár vikur fyrir burð á þurrefnisát, mjólkurframleiðslu og frjósemi kúa. Fylgst var með áti, þunga og holdum, nyt og efnainnihald mjólkur greint, styrkur prógesteróns í mjólk var

mældur. Fóðrunin hafði marktæk áhrif á þurrefnisát fyrir burð, þar sem kraftmeira fóður gaf meira át. Ekki fundust marktæk áhrif tilraunameðferða á þurrefnisát eftir burðinn. Það var ekki marktækur munur á þungabreytingu kúnna milli meðferða. Mjólkurmagn eða efnainnihald mjólkur varð ekki fyrir marktækum áhrifum af mismunandi fóðrun fyrir burð, að undanskildu fitu innihaldi mjólkur sem lækkaði með kraftmeiri fóðrun, og þvagefnisinnihaldi mjólkur sem lækkaði með kraftmeiri fóðrun. Mjaltaskeið hafði marktæk áhrif á nyt, prótein- og mjólkursykurinnihald mjólkur. Þurrefnisát fyrir burð var hærra eftir því sem kýrnar fengu kraftmeira fóður en þær sýndu ekki marktækt meira át eftir burðinn, aukin orka fyrir burð gaf hærra fitu innihald og lægra magn þvagefnis í mjólk, meðferðirnar höfðu ekki marktæk áhrif á frjósemi.

INTRODUCTION

Cows like other mammals have the ability to store nutrients in body tissues during periods of positive nutrient balance which can later be utilized for milk production during periods of negative nutrient balance. In the modern highly bred dairy cow this mechanism is however often used to an extent that causes health problems for the cows (Agenas et al. 2003). Most of the production diseases occur around calving so feeding in the dry period is critical for the cows health and production in early lactation (Ingvartsen 2006; Doepel et al. 2002). Many factors affect feed intake, the most important ones are the animal itself, diet composition and feeding strategies. In late pregnancy and early lactation nutrient demands increase considerably, because of fetal development and then milk production. These requirements call for a coordination of biological processes in tissues, resulting in metabolic changes (Ingvartsen 2006; Harstad 1994). Feed intake is relatively low in the periparturient period (3 weeks prepartum to 3 weeks postpartum) and high producing cows experience negative energy balance and hence, must rely on body reserves. It is a

controversial issue how feeding in the close up dry period should be in order to insure the best outcome in lactation although many experiments have been carried out to improve the understanding of peripartuient dry matter intake and health (Grummer 1995, Dann et al. 1999, Vandehaar et al. 1999, Harðarson and Ingvartsen 2005). It has been recommended by some researchers that dairy producers should try to maximize DMI in the close up dry period to prepare cows to eat more right after calving and thereby reduce the risk of ketosis and other metabolic disorders (Grummer 1995; Minor et al. 1998; Vandehaar, et al. 1999; Doepel, et al. 2002). Others however have suggested that restricted feeding prepartum may be advantageous for increased DMI postpartum and prime the cows for negative energy balance commonly experienced in early lactation. (Holcomb et al. 2001; Agenas, et al. 2003; Douglas et al. 2006).

In the current study cows were fed three diets of different energy levels in the last 3 weeks of the dry period. The article covers results regarding feed intake, liveweight, body condition, milk yield and composition and fertility. A subsequent article will report the effects upon blood parameters and other aspects of health.

MATERIAL AND METHODS

The experiment was performed at the research farm Stóra- Ármót, using 30 Icelandic cows and heifers in individual tie stalls. The animals were blocked by parity (1, 2 and 3+) and randomly assigned with one of three dietary treatments. Complete feeding with equipment from Mullerup A/S Denmark was used, the system was programmed for mixing the diets and feeding the cows. Feeding was *ad libitum*, refusals were weighed three days a week and feeding was adjusted for 5-10% refusals twice a week. Water was available at all times.

Diet composition

Six diet compositions were used in the experiment. Diets were composed of hay, straw, barley and compounded feed in different ratios (Table 1). In the first period, 8-4 weeks prepartum, all cows were fed the same diet. In the second period, 3-0 week's prepartum, cows were on three different dietary treatments. In the third period, 0-3 weeks postpartum, all cows were fed a diet with a composition formulated for fresh cows. In period four all cows were fed a diet with a composition formulated for high yielding cows (Table 1). Samples of the forage were taken once a week and samples of barley and compounded feed were taken every two weeks, to be analyzed for: dry matter, crude protein (CP) using Kjeldahl method, Neutral detergent fiber (NDF) using ANKOM technology on Van Soest method (Van Soest et al. 1991), ashes, dry matter digestion (DMD) using a modified Tilley and Terry method (Tilley and Terry 1963) and minerals. Dry matter was determined twice a week for the forage but once a week for the barley and from the refusals of every diet. Table 2 shows the chemical composition of every feed ingredient.

Weighing and condition scoring

The cows were weighed and condition scored once a week from week 8 before calving until 12 weeks after calving. Cows were weighed after the morning milking, before feeding. Condition scoring was based on a scale from 1-5 where 1 is very thin and 5 very fat. Five spots on the cow were used to estimate the condition; sacrum, lower part of the ribs, wing of ilium, caudal vertebra and ischium. In an article by Bjarnadóttir (2001) the method and scale for condition scoring Icelandic cows is illustrated.

Milk sampling

Cows were milked 2 times a day at 07:00 and 18:00. Milk yield was registered automatically by a SAC milking system at every milking. Energy corrected milk was

calculated according to Sjaunja et al. (1990). Every week, at one morning and one evening milking, milk samples were taken and sent to the research center for the milking industry in Iceland (Rannsóknarstofa mjólkuriðnaðarins) and analyzed for fat, protein, urea, lactose, free fatty acids, casein and somatic cell count. The concentration of progesterone was estimated in milk three times a week, Monday, Wednesday and Friday, using a manual kit from Ridgeway Science in England, starting 14 days after calving. Progesterone in milk samples was measured by a method based on enzymatic degradation of progesterone.

Statistical analysis

Statistical analyses were performed in the computer program MINITAB 14. GLM procedure was used to detect effect of treatment, period and parity on DMI. Treatment-period interaction and treatment-parity interaction was also tested on DMI using GLM procedure.

$$Y_{ij} = \mu + \alpha_i + \gamma_j + \alpha\gamma_{ij} + \varepsilon_{ij}$$

Where Y_{ij} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the over all mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} period, $\alpha\gamma_{ij}$ is the interaction between treatment and period and ε_{ij} is the residual effect. Same model was used to test parity where γ represented parity. DMI in every period separately was tested against treatment to see if there was a significant effect of treatment in individual periods.

Treatment, period and treatment-period interaction was tested on weight and body condition score (BCS). Weight change and BCS change was found by using descriptive statistics.

Treatment, period, parity and all possible interactions between these were tested on ECM, protein, fat, lactose and urea concentration in milk. Ovulation and number of insemination

were all found using descriptive statistics. Effect of treatment was tested on ovulation and insemination frequency using GLM procedure.

RESULTS

Due to differences between actual and expected calving dates, there was some discrepancy between cows for how long they received the experimental ration. The average time on the close up diets was 4.6 weeks. 20 cows received the experimental ration for 3-4 weeks and only 4 cows received the experimental ration for less than 3 weeks. Average time for cows in treatment 4 to receive the experimental ration was 5.6 weeks, for cows in treatment 5 the average time for receiving the experimental ration was 4.5 weeks and cows in treatment 6 received the experimental ration in 3.9 weeks on average.

Dry matter intake

DMI was significantly affected by treatment, periods and parity. When cow weight and weeks from calving were also added to the GLM model, it explained 84% of the total variation in DMI. The effects of treatment and periods on DMI are listed in Table 3. DMI over the whole experiment was about 16% higher in treatment DH than DL. Dry matter intake in the period 8-4 wk prepartum was not significantly different between treatments; DMI in the period 3-0 wk prepartum was significantly different between treatments as DMI increased with increased energy concentration in the diet. DMI postpartum was not significantly different between treatments. There was an interaction between treatment and period: DMI in treatment DL decreased, but in DM and DH it increased, from the period 8-4 week's prepartum to 3-0 week's prepartum. As then could be expected, the increase in DMI after calving was greatest for treatment DL and smallest for treatment DH. This was especially apparent for the heifers (Table 4). Parity affected DMI, i.e. heifers consumed less than older cows (Table 4). DMI increased with increased concentrate level in the

period 3-0 wk prepartum, but treatment did not have a significant effect on DMI in the period 0-3 wk postpartum.

Body weight and condition score

Treatments and periods had a significant effect on cow body weight. Table 5 shows cow weight in all treatments and periods. Table 6 shows weight change during each period from 8 weeks prepartum to 12 weeks postpartum. There is a significant effect of periods but not treatments on weight changes. As for weight, both treatment and period affect BCS significantly (Table 7). There were no significant effects of periods or treatments upon BCS changes (Table 8).

Milk yield and content

The effects of treatments and periods on ECM can be seen in Table 9. There is not a significant difference in ECM between treatments but on average the yield was lowest in treatment DL. There is a significant difference in ECM between periods. There is a significant difference in fat content between treatments and periods, as seen in Table 10. Protein concentration (Table 11) is not significantly different between treatments but between periods. There is not a significant difference in lactose concentration between treatments or periods (Table 12). Treatment and periods affected urea concentration significantly; Table 13 shows the results from urea concentration. Parity affected ECM, protein and lactose significantly.

Fertility

After viewing progesterone concentration in milk we were able to determine time of first ovulation, but only a few cows showed regular oestrus in this period. As table 14 shows there was not a significant difference in ovulation time or number of inseminations needed per conception.

DISCUSSION

Dry matter intake

Different feeding strategies prepartum to improve postpartum DMI and performance have been the major topic of many trials. It has been suggested that prepartum DMI is positively correlated with postpartum DMI and to improve postpartum performance and health, prepartum DMI should be maximized (Grummer 1995). In the current study dairy cows were fed different energy levels in the close up dry period and DMI was measured from 8 weeks prepartum to 12 weeks postpartum. Several researchers have focused on maximizing intake in the dry period (Vandehaar, et al. 1999; Doepel, et al. 2002; Rabelo, et al. 2003), when others have focused on limiting energy intake in the dry period (Douglas, et al. 2006; Agenas, et al. 2003; Holcomb, et al. 2001), all with the same goal of improving DMI postpartum and health. There was no difference in DMI in the period 8-4 weeks prepartum, when all the cows were fed the same diet. DMI in the close up dry period was significantly affected by treatments, where DMI increased with increased energy concentration in diet. DMI postpartum was not significantly affected by prepartum treatments. As parturition approaches energy requirements increase because of a growing fetus and tissues such as the uterus and the mammary gland (Vandehaar, et al. 1999), at the same time as feed intake decreases. By increasing the energy density of the diet prepartum increased energy demands as parturition approaches can better be fulfilled. The results from the current study show that cows in treatment DH had higher DMI prepartum than cows in the other two treatments. Low fiber diets give higher ruminal dry matter digestibility which allows faster rumen digesta evacuation and thereby higher DMI (Rabelo, et al. 2003) which can be advantageous considering the decrease in feed intake that normally occurs as parturition approaches. McNamara et al. (2003) also pointed out that by increasing energy density in the close up dry period, energy intake can be

maintained despite a decline in DMI. Cows in treatment DL ate less in period 3-0 week's prepartum than earlier in the dry period, while the other groups (DM and DH) increased their DMI during this period. Cows in these treatments also had the tendency to eat more postpartum even though it was not significant. Other advantages of including concentrates in the prepartum diet would be to adapt the rumen microflora to high starch diet and to stimulate the growth of rumen papillae (Vandehaar, et al. 1999). Andersen et al. (1999) states that low energy diet can cause degeneration in the rumen epithelium and reduction in VFA absorption capacity. This could have been the case for cows in treatment DL, where they got low levels of concentrate prepartum and had therefore the greatest increase in concentrate levels after parturition. Theoretically, grain supplementation during the late dry period will prepare the cow and her rumen for the consumption of high energy concentrated diet to utilize when lactation commences, but it is unclear to which extent the feeding in late gestation is likely to prepare the rumen for diets after calving (Stockdale 2005; Ingvartsen 2006). According to this, cows in treatment DH and possibly in treatment DM, were better prepared for high concentrate feeding postpartum.

Parity also influences DMI, multiparous cows consume more compared with primiparous cows which compares to others (Keady et al. 2001;Rabelo, et al. 2003). Heifers in treatment DL consumed about 95% of older cows, but the difference was greater in treatments DM and DH were heifers only consumed about 80% of older cows. This difference can possibly be explained by heifers in treatment DL increasing DMI considerably more postpartum than heifers in the other two treatments. Ingvartsen and Andersen (2000) point out that in the first part of lactation intake capacity of heifers is only around 80% of older cows. The difference in DMI between heifers and older cows decreases as parturition approaches which compares to the findings of (Rabelo, et al. 2003).

Body weight and condition score

Body weight or condition was not determining factors when cows were assigned to treatments and therefore there were some differences in body weight between groups from the beginning. Therefore, changes in body weight give us more information on treatment effects than actual weight. All treatment groups show loss in body weight 0-3 weeks postpartum. As parturition approaches the body starts sending out hormonal signals that elicit nutrient mobilization form body reserves. This happens because of the dramatic increase in nutrient demand in the beginning of lactation while DMI lags behind. Although there was no significant difference in body weight change between treatments, DH cows tended to lose more weight than cows in the other treatments. DH cows were the only group that lost body weight in two periods, both 3-0 weeks prepartum and 0-3 weeks postpartum. A possible explanation to this is that the weight of the intestine plays an important role in the total body weight measured. Cows in treatment DH were fed high energy diet which has high digestibility and therefore fast evacuation when cows in treatment DL were fed low energy diet which has lower digestibility and slower evacuation.

Research has shown that thin cows maintain body condition before parturition while fat cows lose condition (Stockdale 2005) and rapid loss of body condition can be associated with a higher incidence of metabolic disorders and fertility problems (Gearhart et al. 1990). Body condition score was significantly different between treatments, but change in BCS was not. It is recommended to keep the condition score at 3.0 – 3.5 (Bjarnadóttir 2001; Gillund et al. 2001). BCS in all treatments was around 3 through out the experiment, BCS loss was less than 0.1, but the greatest BCS gain was 0.24. Gearhart, et al. (1990) found that ideally BCS at drying off should be the same as desired at calving, because losing condition in the dry period increases the odds of health problems. Stockdale

(2005) found that cows that scored lower in body condition consumed less than cows that scored higher, during the transition period. This is opposite to our findings where DH cows consumed most DMI.

Milk yield and content

Feeding diets with three different energy levels prepartum did not affect protein or lactose in milk significantly. This is in agreement with other research. Douglas, et al. (2006) did not find a significant difference in milk production or milk contents in cows fed ad libitum or restricted prepartum. ECM did not differ significantly between treatments. Doepel, et al. (2002) found no effect on milk yield the first 42 days in milking after feeding different energy and protein concentrated diets prepartum. But not all researchers do agree and McNamara, et al. (2003) did find a significantly higher milk yield, protein, fat and lactose concentration after feeding concentrate supplemented diet prepartum. Others have found a significant difference in fat content (Keady, et al. 2001; Holcomb, et al. 2001), which was also the case in this experiment, were cows in treatment DL had significantly more fat in milk than cows in other treatments. It has been speculated that severe fat mobilization in early lactation increases fat content in milk (Agenas, et al. 2003; Keady, et al. 2001). Cows in treatment DL were fed low energy diets and it can therefore been speculated that they had to mobilize more fat than cows in other treatments based on the results from fat content in milk. Urea concentration in milk was significantly affected by treatments and periods, where urea concentration increases with decreased energy in prepartum diet. Kokkonen et al. (2004) found that urea in milk tended to be lower with lower energy feeding, but was significantly higher when concentrate levels were increased quickly. This could explain why cows on the DL treatment that had the greatest increase in concentrate levels postpartum, had the highest urea concentration in milk

Fertility

Fertility parameters found in this experiment, such as average number of AI per conception, are similar to results found from Norwegian cows (Gillund, et al. 2001). Fertility parameters in this experiment were not significantly affected by treatments. Keady, et al. (2001) reported that concentrate supplementation precalving increased day to first progesterone rise, onset of cyclicity and number of AI per conception, but did not affect overall conception rates. Feeding affects body condition and research have shown that body condition can affect fertility (Gearhart, et al. 1990). Gillund, et al. (2001) found no relationship between BCS at calving and reproductive performance, but BCS loss postpartum had a negative effect on reproductive performance. Other health parameters can also affect fertility; Gillund, et al. (2001) found that cows with a history of ketosis were less likely to conceive at first insemination than nonketoic cows.

CONCLUSIONS

DMI prepartum was significantly affected by treatments, feed intake increased with higher energy levels. But this was not significantly maintained into the lactation. Cows in treatment DH had the greatest DMI postpartum indicating an advantage in raising the energy level in prepartum diet. Increased DMI is important for cows to avoid metabolic disorders in the light of their weight loss. Because of a highly concentrated diets prepartum, cows in treatment DH and possibly in treatment DM were better prepared for high concentrate feeding postpartum, which could explain higher DMI postpartum in these groups. ECM was not significantly affected by treatments, but fat content in milk was significantly higher for cows in treatment DL. This indicates that cows in treatment DL had to mobilize more from body reserves than cows in the other treatments and were therefore fed to low energy levels prepartum.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. Svala Sigurgeirsdóttir for daily management of the experiment and Mr. Tryggvi Eiríksson for chemical analysis of feed stuff. Financial support from the Agricultural Productivity Fund is gratefully acknowledged.

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Table 4. Ingredients of diets on a dry matter basis.

Weeks	Treatments	Feed			
		Hay	Straw	Barley	Compounds
8-4	All	60%	40%	-	-
3-0	DL	60%	35%	-	5%
3-0	DM	60%	20%	-	20%
3-0	DH	65%	-	-	35%
0-3	All	54%	-	7%	39%
4-12	All	38%	-	25%	37%

Table 5. Chemical analysis of the feed[1] (in % DM)

Feed	DM	CP	NDF	Ash	DMD	FEm	Ca	P	Mg	K	Na
Hay	30.85	20.21	46.20	9.02	72.81	0.84	0.45	0.40	0.21	2.21	0.09
Straw	79.39	7.27	72.16	6.25	44.0	0.44	0.23	0.11	0.89	0.11	0.12
Barley	49.09	12.50	15.29	2.90		1.14	0.04	0.30	0.11	0.63	0.02
Compounds Prepartum	86.82	20.46	21.79	9.03		1.12	1.44	1.14	0.83	1.04	0.42
Compounds Postpartum	88.05	22.28	17.93	9.80		1.12	2.03	1.15	0.49	1.01	0.48

[1]DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, DMD: dry matter digestion

Table 6. The effects of periods and dietary treatments on dry matter intake (DMI).

	Period				
	Weeks prepartum		Weeks postpartum		
Treatment	**8-4**	**3-0**	**0-3**	**4-12**	**Mean[2]**
DL	8.45	6.44	16.61	21.56	14.57
DM	8.99	9.47	17.56	21.33	16.10
DH	7.67	11.58	17.86	22.17	16.90
Mean[1]	8.39	9.07	17.45	21.57	

P-values: treatment<0.001; periods<0.001; effect of treatment on DMI in period 8-4>0.1; in period 3-0<0.001; in period 0-3>0.1; in period 4-12>0.1.
[1]Mean DMI for each period, all treatments; [2]Mean DMI for each treatment from 8 weeks prepartum to 12 weeks postpartum.

Table 7. The effects of parity on dry matter intake (DMI) 3-0 weeks prepartum and 0-3 weeks postpartum.

	Heifers		Cows in 2. lactation		Cows in 3+ lactation	
	Weeks prepart.	Weeks postpart.	Weeks prepart.	Weeks postpart.	Weeks prepart.	Weeks postpart.
Treatment	**3-0**	**0-3**	**3-0**	**0-3**	**3-0**	**0-3**
DL	6.71	16.98	8.28	17.87	6.61	16.38
DM	8.38	14.75	9.83	19.04	9.97	20.48
DH	10.54	14.33	13.41	20.16	11.24	18.96

P-values: treatment in period 3-0<0.001; treatment in period 0-3>0.1; parity in both periods<0.001

Table 8. The effects of periods and dietary treatments on cow weights.

	Period			
	Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**
DL	485.0	489.3	451.7	450.1
DM	508.4	502.9	466.3	464.0
DH	482.6	486.2	452.3	443.3

P-values: treatment<0.01; periods<0.001

Table 9. The effects of periods and dietary treatments on cow weight change.

	Period			
	Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**
DL	+26.1	+7.2	-13.4	+16.9
DM	+15.9	+13.0	-5.4	+35.3
DH	+45.8	-8.5	-14.9	+22.8

P-values: treatment>0.1; periods<0.05

Table 10. The effects of periods and dietary treatments on cow condition score.

	Period			
	Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**
DL	3.11	3.27	3.02	3.19
DM	3.01	3.07	3.06	3.08
DH	2.96	3.13	3.09	3.06

P-values: treatment<0.01; periods<0.01

Table 11. The effects of periods and dietary treatments on condition score changes.

	Period			
	Weeks prepartum		Weeks postpartum	
Treatment	**8-4**	**3-0**	**0-3**	**4-12**
DL	0.2	0.03	0.38	0.06
DM	0.07	0.09	-0.07	0.14
DH	0.24	0.11	-0.03	0.08

P-values: treatment>0.1; periods>0.1

Table 12. The effects of treatments and periods on ECM (kg d^{-1}).

Treatment	Period	
	0-3 weeks postpartum	4-12 weeks postpartum
DL	25.78	25.82
DM	23.91	28.32
DH	25.82	27.44

P-values: treatment>0.1; periods<0.001; parity<0.001

Table 13. The effects of treatments and periods on milk fat content (%).

Treatment	Period	
	0-3 weeks postpartum	4-12 weeks postpartum
DL	4.12	3.64
DM	3.80	3.49
DH	3.90	3.55

P-values: treatment<0.05; periods<0.001; parity>0.1

Table 14. The effects of treatments and periods on milk protein content (%).

Treatment	Period	
	0-3 weeks postpartum	4-12 weeks postpartum
DL	3.53	3.35
DM	3.53	3.22
DH	3.55	3.29

P-values: treatment>0.1; periods<0.010; parity<0.05

Table 15. The effects of treatments and periods on milk lactose content (%).

Treatment	Period	
	0-3 weeks postpartum	4-12 weeks postpartum
DL	4.63	4.73
DM	4.64	4.68
DH	4.72	4.69

P-values: treatment>0.1; periods>0.1; parity<0.001

Table 16. The effects of treatments and periods on milk urea content (%).

Treatment	Period	
	0-3 weeks postpartum	4-12 weeks postpartum
DL	6.53	7.16
DM	5.74	6.70
DH	5.64	6.65

P-values: treatment<0.001; periods<0.001; parity>0.1

Table 17. Ovulation time, insemination frequency and insemination time for all treatments.

	DL	DM	DH	p-value
Days of first ovulation[1]	29.25	30.40	34.67	0.788
Inseminations[2]	1.63	1.64	1.70	0.978
Conception rate[3] (%)	50	63	50	

[1]Days postpartum
[2] Number of inseminations per conception
[3] After 1. Insemination

Effect of feed in the dry period of Icelandic dairy cows on blood parameters and liver condition

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ABSTRACT

Thirty Icelandic cows and heifers in individual tie stalls were used to examine the effects of three dietary energy levels fed 3-0 week's prepartum on metabolic status around parturition. Blood and liver samples were collected. Glucose, BHB, NEFA, GLDH, AST, GGT, ICDH were analyzed from blood and liver fat was estimated under a microscope. Ketolac® test was used to estimate the level of BHB in milk. Treatments did not affect results from Ketolac® test or liver fat estimation significantly. Glucose concentration in blood increased significantly with increased energy level, there was also a significant effect of periods and parity. Treatment did not affect BHB or NEFA concentration significantly. BHB concentration was highest in the first three weeks postpartum and heifers had lower BHB concentration than older cows. NEFA concentration was highest around parturition. Treatments affected GLDH enzyme concentration significantly, GLDH increased with increased energy level in diet. Treatments did not affect AST, GGT or ICDH significantly. Although only a few blood parameters were significantly affected by treatments, the results indicate that the risk of developing production diseases was greater for cows receiving the highest energy level prepartum.

Keywords: Non esterified fatty acids, β-hydroxybutarate, fatty liver, liver enzyme.

Abbreviation key: DL = low energy level; **DM** = medium energy level; **DH** = high energy level; **BHB** = β-hydroxybutyrate; **NEFA** = Non-esterified fatty acids; **GLDH** = Glutamate dehydrogenase; **AST** = Aspartat aminotransferase; **GGT** = Gamma glutamyl transferase; **ICDH =** Isocitrate dehydrogenase

Yfirlit

Markmið rannsóknarinnar var að athuga áhrif mismunandi orkuríkrar fóðrunar seinustu þrjár vikurnar fyrir burð á efnaskipti líkamans í kringum burðinn. Blóð- og lifrarsýnum var safnað. Glúkósi, BHB, NEFA, GLDH, AST, GGT og ICDH var greint í blóði og fita í lifur metin undir smásjá. Auk þess sem BHB í mjólk var greind. Meðferðirnar höfðu ekki marktæk áhrif á niðurstöður mjólkurprófsins eða fitu í lifur. Glúkósi jókst marktækt í blóði eftir því sem kýrnar fengu orkuríkara fóður, einnig höfðu tímabil og mjaltaskeið marktæk áhrif. Meðferðirnar höfðu ekki marktæk áhrif á magn BHB eða NEFA í blóði. Magn BHB í blóði var mest fyrst eftir burðinn og einnig höfðu eldri kýr meira BHB í blóði en kvígur. NEFA í blóði var mest í kringum burð. Meðferðirnar höfðu marktæk áhrif á GLDH magn, sem jókst með aukinni orku í fóðri. Meðferðirnar höfðu ekki marktæk áhrif á AST, GGT og ICDH. Meðferðirnar höfðu ekki marktæk áhrif á alla þá heilsufars þætti sem skoðaðir voru en gáfu hinsvegar vísbendingar um að kýr sem fengu mesta orku fyrir burð hefðu verið í meiri hættu á að fá framleiðslusjúkdóma.

INTRODUCTION

The metabolism of dairy cows around calving is under a lot of strain, which often results in metabolic disorders and production diseases. The period from three weeks prepartum to three weeks postpartum, the periparturient or transition period, is critical in this respect. Cows can easily put on condition in the first half of the dry period but as parturition approaches dry matter intake (DMI) decreases. At the onset of lactation the

demands for energy and metabolic precursors increase faster than DMI can deliver with the consequence that a negative energy balance is unavoidable.

Inadequate feeding of high yielding cows increase metabolic stress in the beginning of lactation. Therefore, diseases will often appear in the first days or weeks of lactation. Production diseases such as fatty liver, ketosis, rumen acidosis and displaced abomasum are to a large extent related to problems in feeding and management (Ingvartsen 2006). The feeding management in the dry period has been an issue of considerable controversy. Dairy producers have been advised to maximize dry matter intake (DMI) close to parturition to prepare for higher DMI after calving, which would reduce metabolic disorders (Grummer 1995,Minor et al. 1998,Vandehaar et al. 1999,Doepel et al. 2002). Others have found that the energy density of the prepartum diet has a minor influence on postpartum metabolic status, compared with the energy density of diets fed during the first three weeks postpartum (Rabelo et al. 2005). Douglas et al. (2006) suggested that feeding management should promote vigorous appetites postpartum with a faster increase of DMI. Cows fed restrictive diets in the dry period may perform well postpartum if they are offered a high quality diet *ad libitum* after parturition. On the other hand, cows fed excessively prepartum may mobilize more of their body reserves and suffer a prolonged negative energy balance postpartum (Keady et al. 2001,Holtenius et al. 2003). Many metabolic changes are unavoidable around parturition but good feeding management can make it possible to minimize problems related to excessive metabolic changes.

Feeding management in the last 3 weeks prepartum is the main topic of this study. The effects of feeding Icelandic cows diets with three different energy levels during this period were investigated. This paper covers results regarding liver condition and blood parameters. A companion paper (Óðinsdóttir, et al. 2009) reports the effects of the same experimental treatments upon feed intake, yield, milk composition and fertility.

MATERIAL AND METHODS

The experiment was performed at the research farm Stóra Ármót, using 30 Icelandic cows and heifers in individual tie stalls. The animals were blocked by parity (1, 2 and 3+; where 1, 2 and 3+ is, respectively, 1., 2., and ≥3. parity) and randomly assigned with one of three dietary treatments. Complete feeding with equipment from Mullerup A/S Denmark was used, the system was programmed for mixing the diets and feeding the cows. Feeding was *ad libitum*, refusals were weighed three days a week and feeding was adjusted to 5-10% refusals twice a week. Water was available at all times.

Diet composition

Six diet compositions were used in the experiment. Diets were composed of hay, straw, barley and compounded feed in different ratios (Table 1). In the first period, 8-4 week's prepartum, all cows were fed the same diet. In the second period, 3-0 week's prepartum, cows were on three different dietary treatments. In the third period, 0-3 weeks postpartum, all cows were fed a diet with a composition formulated for fresh cows. In period four, 4-12 weeks postpartum, all cows were fed a diet with a composition formulated for high yielding cows (Table 1). Samples of the forage were taken once a week and samples of barley and compounded feed were taken every two weeks, to be analyzed for: dry matter, crude protein (CP) using Kjeldahl method, neutral detergent fiber (NDF) using ANKOM technology on Van Soest method (Van Soest et al. 1991), ashes, dry matter digestibility (DMD) using a modified Tilley and Terry method (Tilley&Terry 1963) and minerals. Dry matter was determined twice a week for the forage but once a week for the barley and from the refusals of every diet. Table 2 shows the chemical composition of every feed ingredient.

Liver sampling

For assessing the condition of the liver 23 needle biopsies were taken from the liver of 22 cows around calving. Samples were taken in the period from 3 days prepartum to 8 days postpartum, using puncture biopsies (Hughes 1962). The area between the 11th and 12th ribs on the right side was clipped and disinfected. Then the area was anaesthetized by using a local anaesthetic lidocain. Small incision was made parallel to the ribs and the needle directed towards the liver. The samples were at first stored in liquid nitrogen and then fixed in 10% buffered formalin. After that they were embedded in paraffin, sectioned at 3 µm thickness and stained with hematoxylin and eosin. Cellular fat content was rated using two methods: firstly, manually under a microscope where samples were rated from 0 to 5 (Steen et al. 1992), 0 being normal liver cells and 5 almost all cells having filled vacuole cytoplasm and pyknotic nucleus; and secondly by using the computer program Leica QWin. The computer program calculated total area of fat in sample, mean area of fat in sample and number of fat features in sample.

Blood sampling

Blood sampling was made once a week, from 4 weeks before expected calving until 6 weeks after calving and then 8, 10 and 12 weeks after calving. Blood sampling was always performed at the same time a day, in the morning after milking and feeding. Blood was drawn from the coccygeal vessel under the tail into a lithium heparin tube, to prevent coagulation. The tubes were put in ice-bath to stop metabolic reactions. After centrifugation, plasma samples were put into a freezer and stored at -20°C until they were analyzed for glucose, BHB, NEFA, AST, GGT, GLDH, and ICDH. Blood plasma glucose, AST, and GGT were determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650). NEFA were determined using the Wako, NEFA C ACS-ACOD assay method. BHB was determined as an increase in absorbance at 340 nm

due to the production of NADH, at slightly alkaline pH in the presence of β-OH-butyrate dehydrogenase. Sample blank was included. The method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by (Harano et al. 1985). GLDH activity was quantified in a kinetic, colorimetric assay according to (Schmidt&Schmidt 1995). ICDH was determined in a kinetic, colorimetric assay using isocitrate as substrate and $NADPH_2$ as response parameter.

All analyses were performed using an autoanalyzer, ADVIA 1650 ® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA). Precision of all parameters was below 2 CV% (intra assay) and 4 CV% (inter assay). Chemicals analyzed from blood plasma are listed in table 3 and a short description of their usage is given.

Ketolac® test

Ketolac stick test (Sanwa Kagaku Kenkyusho co.Ltd., Japan) was used to detect the level of β-hydroxy-butyrate (BHB) in milk. The stick contains β-hydroxy-butyrate-dehydrogenase which converts β-hydroxy-butyrate (BHB) and NAD^+ into acetoacetic acid (ACA) and NADH. At the same time NADH reduces the Nitrotetrazolium-blue (NTB) contained in the stick to the purple Formazan. By comparing the intensity of the colour change on the stick with a colour chart it is possible to determine β-hydroxy-butyrate (BHB) concentration in milk. The test was performed 3 times per cow, 1, 2 and 3 weeks after calving.

Statistical analysis

Statistical analyses were performed in the computer program MINITAB 15 (Minitab, Inc. Pennsylvania, USA). GLM procedure was used to detect effect of treatment, period and parity on glucose, AST, GGT, BHB, GLDH, ICDH and NEFA. Treatment-period and treatment-parity interactions were also tested.

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \delta_k + \alpha\gamma_{ij} + \alpha\delta_{ik} + \varepsilon_{ijk}$$

Where Y_{ijk} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} period, δ_k is the effect associated with the k_{th} parity, $\alpha\gamma_{ij}$ is the interaction between treatment and period, $\alpha\delta_{ik}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

GLM procedure was also used to determine whether treatment, parity or body condition score (BCS) affected BHB in milk.

$$Y_{ijkl} = \mu + \alpha_i + \gamma_j + \delta_k + \eta_{l+} \alpha\gamma_{ij} + \alpha\delta_{ik}\ \varepsilon_{ijk}$$

Where Y_{ijkl} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} week (1,2,3), δ_k is the effect associated with the k_{th} parity, η_l is the random effect associated with the l_{th} body condition score, $\alpha\gamma_{ij}$ is the interaction between treatment and week, $\alpha\delta_{ik}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

The relationship between BHB measured in milk and BHB measured in blood was tested by using Regression procedure.

When estimating the liver condition, GLM procedure was used where treatment and parity, were tested against the liver parameters, total area of fat, mean area of fat, number of fat features and fat content rated.

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \delta_k + \alpha\gamma_{ij} + \alpha\delta_{ik}\ \varepsilon_{ijk}$$

Where Y_{ijk} is the record of the j_{th} animal assigned to the i_{th} treatment (DL, DM, DH), μ is the overall mean, α_i is the effect associated with the i_{th} treatment, γ_j is the effect associated with the j_{th} parity, $\alpha\gamma_{ij}$ is the interaction between treatment and parity and ε_{ij} is the residual effect.

Weight, BCS, ECM and liver enzymes were also tested individually against the liver parameters.

RESULTS

Due to differences between actual and estimated calving dates, there was some discrepancy between cows for how long they received the experimental ration. The average time on the late dry period diets (DL, DM and DH) was 4.6 weeks. 20 cows received the experimental ration for 3-4 weeks and only 4 cows received the experimental ration for less than 3 weeks. Average time for cows in treatment DL to receive the experimental ration was 5.6 weeks, for cows in treatment DM the average time for receiving the experimental ration was 4.5 weeks and cows in treatment DH received the experimental ration for 3.9 weeks on average.

Liver samples

Treatments did not have a significant effect on total area of fat in sample, mean area of fat in sample or number of fat features as estimated by the Leica Qwin computer program. Treatments did not affect cellular fat rating under a microscope; Table 4 shows the effect of treatments on liver parameters. Parity had a significant effect on total area of fat in sample, mean area of fat in sample and number of fat features found in sample in the computer program. Parity also affected fat rating under a microscope significantly. Liver fat increased with increased number of parturitions as seen in Table 5. Average weight in the last three weeks prepartum affected all liver fat parameters positively and significantly ($P<0.001$. Mean body condition score in the last three weeks prepartum also had a positive relationship with mean area of fat ($P<0.05$), cellular fat rating under a microscope ($P<0.001$). Mean ECM in the first 12 weeks in lactation was positively related to total area of fat ($P<0.001$), mean area of fat ($P<0.001$) and number of fat features ($P<0.001$) found by the computer program. The concentrations of all liver enzymes (AST, GGT, GLDH, ICDH) increased significantly ($P<0.005$) with increased fat in liver.

Blood samples

Plasma glucose concentration increased significantly with increased energy concentration of the diet 3-0 weeks prepartum (Table 6). There was also a significant effect of periods and parity, where glucose concentration reached a minimum in the first period postpartum and heifers had higher glucose concentration than multiparous cows. There was an interaction between treatment and period because of lower plasma glucose concentration in period 3-0 wk prepartum for DM cows, when the other two treatments increased plasma glucose concentration as parturition approached. Plasma glucose concentration was lowest in the period 0-3 wk postpartum for all treatments, and remained lower in the second postpartum period (4-12 wk) than in the dry period. The greatest drop around parturition was for DH cows. Heifers had higher plasma glucose concentration than multiparous cows.

There was no significant effect of treatments on plasma BHB concentration, but there was a significant effect of period and parity. Plasma BHB concentration is higher postpartum than prepartum, and by far the highest first after parturition (0-3 wk postpartum) (Table 7). Plasma BHB concentration was lower for heifers than for multiparous cows.

Treatments affected plasma NEFA concentration significantly in the period 0-3 weeks postpartum and there was a significant effect of periods. Plasma NEFA concentration was highest in the periods around parturition (3-0 wk prepartum and 0-3 wk postpartum (Table 8). There was a trend for plasma NEFA concentration to increase with increased parity. There was an interaction between treatment and period. Cows in treatment DM had the highest NEFA concentration 3-0 weeks prepartum but cows in DL and DH showed highest NEFA concentration in period 0-3 weeks postpartum.

There was no significant effect of treatments on plasma AST concentration, but the effect of periods was significant (Table 9). Plasma AST concentration increased after parturition and remained higher postpartum than in the dry period.

Plasma GGT concentration was significantly affected by period, parity and there was an interaction between treatment and parity. Plasma GGT concentration decreased from the period 8-4 wk prepartum to the period 3-0 wk prepartum and then increased in the period 0-3 wk postpartum and even more in the period 4-12 wk postpartum (Table 10). Heifers had the lowest plasma GGT concentration and cows in lactation 3+ had higher concentration than cows in their second lactation.

Plasma GLDH concentration was significantly affected by treatment and period. Plasma GLDH concentration was positively correlated with energy concentration in prepartum diet, where DL cows had the lowest concentration and DH cows the highest (Table 11). Plasma GLDH concentration was higher postpartum than prepartum and actually increased from the period 8-4 wk prepartum to the period 4-12 wk postpartum. There was a trend for the heifers to have lower plasma GLDH concentration than multiparous cows.

Plasma ICDH concentration was significantly affected by period and parity. Plasma ICDH concentration was higher postpartum than prepartum and the concentration was positively correlated with parity (Table 12).

Ketolac® test

There was not a significant difference in BHB concentration in milk between treatments, weeks or parity (Table 13). There was a significant correlation between BHB concentration measured in milk and BHB concentration measured in blood with a P-value<0.001 and r^2=32.7%. The regression equation is: BHB in blood = 1.03 + 0.323 BHB in milk.

DISCUSSION

In the present study, the effects of different feeding strategies prepartum on health parameters, such as glucose, NEFA, BHB and liver enzyme concentration in blood and liver fat, were examined. In a companion paper (Óðinsdóttir et al., 2009, submitted) DMI and production performance in the same experiment were studied. Chemical analyses of status parameters in the blood can give a good picture of the metabolic status of the whole body. Changes in plasma concentration of glucose, NEFA and BHB are consistent with energy deficiency and adipose tissue mobilization which occurs in the first weeks postpartum (Reid&Roberts 1982).

All cows start to mobilize fat prepartum and NEFA concentration in blood rises as parturition approaches (Bertics et al. 1992). This corresponds with the results of the present experiment. It can be used as an indicator of fatty liver when NEFA plasma concentration does not decline shortly after parturition, here NEFA were at normal levels in week 4-12 for all treatments. Dietary treatments did not have a significant effect on NEFA concentration in blood plasma throughout all periods. But treatments did affect plasma NEFA concentration significantly in the period 0-3 weeks postpartum. Cows in treatment DH had the highest concentration postpartum and cows in treatment DL showed the lowest concentration. These results are consistent with the results of Vandehaar et al. (1999) where prepartum dietary treatment did not alter plasma NEFA, but there was a trend for postpartum plasma NEFA to be greater in cattle fed the high energy diet before calving than those fed the low energy diet. Rabelo et al. (2005) found on the other hand that cows fed high energy diets prepartum tended to have lower plasma NEFA postpartum than cows fed low energy diets prepartum.

With severe lipid mobilization triglycerides start to accumulate in the liver and fatty liver develops. Fatty liver does not necessarily involve liver dysfunction and can return to

normal structure and function once the metabolic status is corrected (Jubb et al 1993; seen in(Harðarson&Ingvartsen 2005)). Feeding diets of three different energy concentrations prepartum did not affect fat content in liver significantly. There was not much fat in the liver samples in general and seemed to be equally distributed between treatments. Average weight and BCS three weeks prepartum was significantly and posetively related to fat content in liver. This corresponds with greater postpartum DMI depression and greater fat mobilization seen in fat cows (Reid&Roberts 1982,Lacetera et al. 2005). ECM during the first 12 weeks of lactation was significantly and positively associated with liver fat content. This is also consistent with Reid&Roberts (1982) who say that high yielding cows are at greater risk of fat mobilization. It is therefore important to avoid excessive fatness of cows at calving and try to maximize energy intake after calving to meet requirements. In the present study, parity affected liver fat content significantly, i.e. liver fat increased with increased number of parturitions. Heifers showed higher plasma NEFA concentration prepartum than older cows, but NEFA concentration in heifers decreased sooner and faster after parturition than in older cows. Over all, heifers showed lower NEFA concentration than older cows. This corresponds with the findings of others (Vandehaar et al. 1999), and can possibly be explained by the fact that heifers produce less milk than older cows which makes it easier to maintain positive energy balance postpartum, or at least minimize the effects of a negative energy balance. Rabelo et al. (2005) pointed out that multiparous cows are more likely to be overconditioned than heifers and are therefore predisposed to fatty acid mobilization from adipose tissue which contributes to a greater TG accumulation in liver postpartum.

Postpartum changes in blood concentration of glucose, NEFA and BHB are consistent with the energy deficiency and adipose tissue mobilization which commonly occur in early lactation (Reid&Roberts 1982). Decreased glucose concentration postpartum

is also related to slow increase in DMI and thereby propionate absorption compared to the fast increase in glucose requirement for milk synthesis (Doepel et al. 2002). BHB concentration is a better long term indicator of feeding than glucose concentration which changes quickly when feeding is changed. Our results showed significant increase in plasma glucose concentration prepartum with increased concentrate in feed which is consistent with the results of Rabelo et al. (2005) who found increased glucose concentration in blood when feeding high energy diet compared to low energy diet. Research has shown that postpartum glucose concentration is unaffected by different energy concentration prepartum (Doepel et al. 2002,Rabelo et al. 2005,Douglas et al. 2006). In the present study, plasma glucose concentration drops postpartum in relation to increased energy requirements. Cows in treatment DH showed the greatest drop in glucose concentration around parturition and the lowest concentration of glucose in blood the first weeks postpartum. The glucose concentration then increases again when energy metabolism has stabilized. Younger cows have a significantly higher glucose concentration in blood plasma. That can be explained by less production and thereby a lower energy deficiency, DMI can better support their production. Other research has shown the same results (Rabelo et al. 2005).

BHB concentration is also a good indicator of energy balance. The concentration of BHB in blood indicate the level of ketosis and in a study of Geishauser et al. (2000) cows with more than 1.4 mM BHB in blood serum had a significantly higher risk of developing clinical ketosis than cows with less than 1.4 mM of BHB in blood serum. In a study by Enjalbert et al. (2001) cows with blood BHB concentration over 1.2 mM were defined as having subclinical ketosis. Subclinical ketosis can affect milk production and reproduction. It is therefore important to treat subclinical ketotic cows before the condition increases and causes marked effect on the performance (Geishauser et al. 1998). Cows in this experiment

showed BHB concentration in the period of three weeks postpartum from 1.1 to 1.4 mM. Only five cows in this experiment showed signs of ketosis, and they were from all treatments. There was not a significant effect of treatments on BHB concentration in blood or in milk. Cows in treatment DH did however show the highest BHB concentration in blood in the first three weeks postpartum (1.4 mM) and cows in treatment DL showed the lowest BHB concentration in blood postpartum (1.1 mM). BHB concentration rises around parturition and reaches a maximum in the period of the first three weeks postpartum. This is because of the high energy requirements in the beginning of lactation and the cows have to use this way for energy resources. Heifers showed lower BHB concentration than older cows, corresponding to glucose concentration results.

Routine monitoring of ketone bodies in blood is not an option because blood sampling is not easy for farmers. There was a significant correlation between BHB concentration measured in milk and BHB concentration in blood in this experiment. Detection of milk ketones with the use of Ketolac test can therefore be useful in a routine monitoring to detect subclinical ketosis postpartum because of its simplicity. It has even been shown that Ketolac test is more sensitive for detecting subclinical ketosis than other tests available (Geishauser et al. 1998). To detect subclinical ketosis cows should be tested regularly during the first month postpartum, examining once a week may provide good insight into changes of the ketosis status (Geishauser et al. 2000). Subclinical ketosis is most often observed in weeks 2 and 3 in lactation (Enjalbert et al. 2001).

All liver enzymes analyzed where found in higher concentrations postpartum. As parturition approaches, indicators of an insufficient energy supply, such as NEFA and BHB concentration in blood increase. This increases stress on the liver, the same thing applies first after parturition when the cows suffer from a negative energy balance. It can therefore be expected that liver enzymes can more easily escape from the liver postpartum

and liver enzyme concentration in blood rises. The liver enzymes that are analyzed in blood plasma are good indicators of liver integrity. These enzymes start to diffuse from the liver into the blood when the liver is under pressure and liver function is impaired. This is consistent with the results in this experiment; concentration of all liver enzymes measured was affected by periods. Liver enzyme concentration was always higher postpartum than prepartum. Treatments only affected concentration of the enzyme GLDH significantly, DH cows had highest GLDH concentration in blood and DL cows had the lowest concentration. The enzyme GLDH participates in insulin metabolism in the body, this enzyme could be affected significantly be treatments because of the significant affect be treatments on glucose. Parity affected GGT and ICDH concentration significantly; heifers had lower liver enzyme concentration in blood than multiparous cows. This is all consistent with the fact that depressed energy balance and therefore more stress on the liver increases liver enzyme concentration in blood. Concentration of liver enzymes in blood was significantly higher when liver fat content was rated or calculated higher. It is therefore possible to assume that the concentration of liver enzymes in blood can be used for diagnosing fatty liver, but further research is needed.

CONCLUSIONS

Liver fat was not significantly different between treatments. In general there was little liver fat infiltration in these cows. Heifers had significantly less liver fat than older cows which could be expected. They can more easily support their production and maintain better metabolic status. Liver enzyme concentration in blood can possibly be used as an indicator of fatty liver. Not all blood parameters were significantly affected by treatments, but they indicated that cows in treatment DH were in greater danger of developing production diseases than cows in the other treatments.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. Svala Sigurgeirsdóttir for daily management of the experiment and Mr. Tryggvi Eiríksson for chemical analysis of feed stuff. We are also thankful to Sverrir Harðarson for embedding the liver samples on glass and Jónína Jóhannsdóttir with Matís for assisting with the computer program Leica QWin. Financial support from the Agricultural Productivity fund is gratefully acknowledged.

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Table 18 Composition of diets.

Weeks	Treatment	Feed			
		Hay	Straw	Barley	Concentrate
-8 to 4	All	60%	40%	-	-
-3 to 0	DL	60%	35%	-	5%
-3 to 0	DM	60%	20%	-	20%
-3 to 0	DH	65%	-	-	35%
0 to 3	All	54%	-	7%	39%
4 to12	All	38%	-	25%	37%

Table 19 Dry matter (DM) content in % of wet weight and chemical analysis of the feed, in % of DM.

Feed	DM	DMD	CP	NDF	Ash	Ca	P	Mg	K	Na
Hay	30,85	72,81	20,21	46,20	9,02	0,45	0,40	0,21	2,21	0,09
Straw	79,39	44,0	7,27	72,16	6,25	0,23	0,11	0,89	0,11	0,12
Barley	49,09		12,50	15,39	2,90	0,04	0,30	0,11	0,63	0,02
Compounds prepartum	86,82		20,46	21,79	9,03	1,44	1,14	0,83	1,04	0,42
Compounds postpartum	88,05		22,28	17,93	9,80	2,03	1,15	0,49	1,01	0,48

[1)]Calculated energy value (FE_m) is per kg DM.

Table 20 Parameters analyzed in blood plasma and a short description of their usage.

Chemical name	Description
Glucose	Monosaccharide; to estimate energy status of the body
BHB	β-hydroxybutyrate; a ketonic substrate, to estimate energy status
NEFA	Non-esterified fatty acids; to estimate energy status of the body
AST	Aspartate aminotransferase; mainly a liver enzyme
GGT	Gamma glutamyl transferase; mainly a liver enzyme
GLDH	Glutamate dehydrogenase; mainly a liver enzyme
ICDH	Isocitric dehyrogenase; mainly a liver enzyme

Table 21 The effect of treatments on total and mean area of fat in liver samples, number of fat features in sample found in the computer program Leica Qwin (μm^2); and cellular fat rating under a microscope (0-5).

Treatments	Total area of fat in sample	Mean area of fat in sample	Number of fat features in sample	Cellular fat rating
DL	7,909,819	261,183	31.208	1.222
DM	9,667,750	227,477	37.000	1.333
DH	14,903,194	292,158	46.167	1.000

P-values: Total area > 0.1; Mean area > 0.1; Fat features > 0.1; Fat rating > 0.1

Table 22 The effect of parity on total and mean area of fat in liver samples, number of fat features in sample found in the computer program Leica Qwin (μm^2); and cellular fat rating under a microscope (0-5).

Parity	Total area of fat in sample	Mean area of fat in sample	Number of fat features in sample	Cellular fat rating
Heifers	3,225,486	240,642	13.208	0.333
Cows in 2. lactation	9,103,917	219,516	37.000	1.333
Cows in 3 + lactation	20,151,361	320,660	46.167	1.888

P-values: Total area < 0.001; Mean area < 0.05; Fat features < 0.001; Fat rating < 0.001

Table 23 Glucose (mM) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	**8-4**	**3-0**	**0-3**	**4-12**			
DL	3.44	3.49	3.12	3.36	3.62	3.20	3.23
DM	3.52	3.51	3.22	3.42	3.67	3.31	3.28
DH	3.67	3.82	3.07	3.44	3.83	3.31	3.36

P-values: treatment<0.05; periods<0.001; parity<0.001; treatment x period<0.01

Table 24 β hydroxyl butyrate (BHB) (mM) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	**8-4**	**3-0**	**0-3**	**4-12**			
DL	0.750	0.748	1.163	0.958	0.792	0.919	1.002
DM	0.747	0.690	1.212	0.927	0.752	0.903	1.026
DH	0.692	0.739	1.406	0.913	0.785	1.093	0.935

P-values: treatment>0.1; period<0.001; parity<0.001

Table 25 Non esterified fatty acids (NEFA) (μ ekv. L^{-1}) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	**8-4**	**3-0**	**0-3**	**4-12**			
DL	154	236	243	117	170	174	218
DM	161	309	238	104	191	228	190
DH	157	280	358	119	194	242	249

P-values: treatment>0.1; periods<0.001; parity<0.1; treatment x period<0.05; treatments in period 0-3 weeks postpartum<0.01

Table 26 AST (U L^{-1}) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	8-4	3-0	0-3	4-12			
DL	58	58	95	87	69	70	84
DM	63	62	78	85	71	74	72
DH	66	62	79	59	78	69	72

P-values: treatment>0.1; periods<0.001; parity>0.1

Table 27 Gamma glutamyl transferase (GGT) (U L^{-1}) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	8-4	3-0	0-3	4-12			
DL	19	17	22	24	20	21	20
DM	18	18	19	21	17	18	22
18,24DH	20	18	20	22	18	19	24

P-values: treatments>0.1; periods<0.001; parity<0.001; treatment x parity<0.001

Table 28 Glutamate dehydrogenase (GLDH) (U L^{-1}) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	8-4	3-0	0-3	4-12			
DL	9	10	16	25	11	16	17
DM	13	12	15	23	12	17	17
DH	22	15	27	29	23	20	27

P-values: treatments<0.01; periods<0.001; parity<0.1; period 0-3 weeks postpartum<0.01; treatments in period 0-3 weeks postpartum<0.01

Table 29 Isocitric dehydrogenase (ICDH) (U L^{-1}) concentration in blood by treatments, periods and parity.

	Period				Parity		
	Weeks prepartum		Weeks postpartum		Heifers	Cows 2. lactation	Cows 3+ lactation
Treatment	8-4	3-0	0-3	4-12			
DL	59	64	92	87	71	76	79
DM	67	68	86	90	65	83	86
DH	65	65	81	92	73	77	78

P-values: treatments>0.1; periods<0.001; parity<0.01

Table 30 BHB according to Ketolac test in weeks 1, 2 and 3 postpartum for the different treatments.

Treatment	Weeks postpartum		
	1	2	3
DL	2.3	1.2	0.5
DM	1.2	1.8	1.5
DH	1.3	2.0	2.2

P-values: treatment>0.1; weeks>0.1; parity>0.1; BCS<0.01